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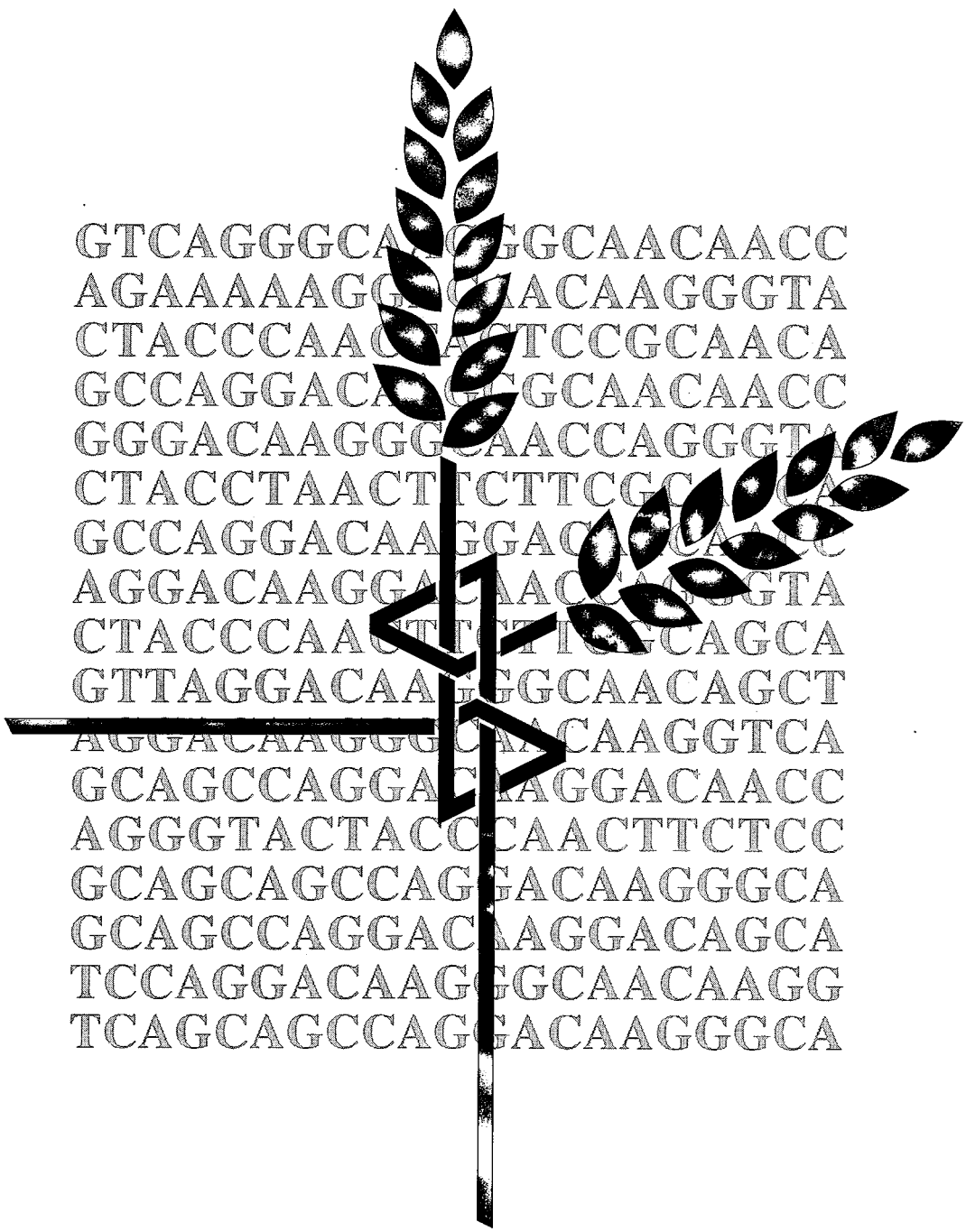

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Review

Perennial and wild *Triticeae* in Japan, description and a short review on process of studies, investigations and results.

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Introduction

Species of the tribe *Triticeae*, *Gramineae* are widely distributed throughout the Japanese islands. However, since the islands are located far away from the center of the origin of the tribe, which is supposed to be the western part of the Asian continent, characteristics are expected to be differently differentiated. Besides remote geographical location, the Japanese climate might have contributed to their specialization. Nevertheless, the species found in Japan are thought to be very important. Here, I will describe as a short review the feature of the Japanese *Triticeae* species, regarding taxonomy and cytogenetics conducted at universities and institutions of Japan by 1990. I summarize especially, studies made by the Japanese researchers, reported mainly in Japanese language in domestic publications (research papers, reports, abstracts). In addition, the author wants to introduce ideas and propositions concerning taxonomy and cytogenetics results of investigations conducted for years.

Studies on the tribe *Triticeae* species in Japan would be divided into at least four different periods.
1. Identification of scientific names with Linnaean classification system in 1800's.
2. Establishment of the *Triticeae* flora in Japan after 1900~1940.
3. Interspecific cross hybridization, chromosome pairing studies in relation to the classification.
4. Hybridization studies using embryo-rescue, detailed analysis of the F_1 and progenies.

According to a recent system, Japanese *Agropyron*, which was often treated as *Roegneria*, is included in *Elymus*. Nevertheless, for avoiding confusion, I will use the traditional species name mainly following Ohwi (1984), and I shall start with plant geographical point of view that will give some idea for the environment of the regions where the indigenous species were distributed.

I. Location of Japanese islands

Present-day Japan extends roughly from N45° of Hokkaido to the south N24° near the Tropic of Cancer. In comparison with European countries, Japan is located relatively south, equivalent to the Mediterranean area and to North Africa. The main parts of Japan belong to the warm temperate region. Because of the relative southern location, differences in the day length between summer and winter are less wide as compared with Europe. At Tokyo (N35° 41') the summer day length is not as long as in many European cities.

II. General tendency of the climate of Japan and vegetation

Although the climate of the Japanese islands (archipelago) is generally mild and warm with predominating oceanic climate, it tends not to be always true. The Japanese archipelago is characterized by monsoon climate and is located in the area where cold and warm fronts are extended and are often hovering overhead. Especially the days around the summer solstice, it is a period of heavy precipitation called "Baiu" (the rainy spell in early summer) that lasts usually more than 40 days. After the heavy rainy season passes, except in the alpine region and Hokkaido, comes a tropical type of summer with humid and high air temperature. Islands are

often hit by a typhoon, which is accompanied by heavy precipitation and strong wind of 25 meters per second, or higher. During the period of "Baiu" and following summer, introduced foreign plants not adapted tend to die out because of suffering by high temperature, less sunshine and high humidity, which reaches more than 70% up to 100%. Introduced *Triticeae* lines are not exceptions; it is usual to have severe difficulties in cultivation in experiment fields and often die without seed set.

Winters are severe in the different ways. Strong wind from Siberia, locating north-west across the Japan Sea, brings cold and the humid air when passes through on a warm sea current then hits high central backbone mountains and produces deep snow accumulation of meters for the mountain slope-side front of the Japan Sea, while it produces cold and dry weather for the Pacific Ocean side. It will be understood that, different from European countries, the *Triticeae* species must pass through both vegetative and reproductive phases within relatively short period by beginning June in Japan. However, this indicates, if we see from the different point of view, the species distributed in Japan have characters that are tolerated both by unfavorable seasons with typical Baiu, and are thought to be unique genetic resources.

Wolfe (1979) reported on the forests of Eastern Asia. The kind of forest that covers from the central Honshu to Shikoku and southern Kyushu, except high mountain area, was termed the Notophyllous Broad-leaved Evergreen forest (oak-laurel forest). Then in the southern area next to there the Ryukyu Islands are covered by paratropical rain forests. Forests change to north in the following order: that is the Mixed Broad-leaved Evergreen and Coniferous, Mixed Mesophytic, Mixed Northern Hardwood, and Mixed Coniferous.

Vegetation areas of "Notophyllous Broad-leaved Evergreen" and "the Mixed Broad-leaved Evergreen and Coniferous" show almost equally the same with a forest termed "the warm-temperate evergreen (lucidophyll) forest" by Kira (1991), who considered the importance of the warmth index and high precipitations. Although I will keep in mind to discuss this in a separate paper in detail, there is some parallel distribution between the forest type and the species of *Triticeae* (For the Japanese species see Table 1 in a later chapter) showing a kind of habitat segregation.

III. A view on the Japanese *Triticeae* species

Triticeae species are found throughout Japan, except such as the subtropical territories of Sakishima Rettou (located south-west of the Okinawa Islands) and Ogasawara archipelago (including Iwoujima) etc. In the rest of the land, the kinds of the species distributed differ depending on the areas. It is thought that these differences are mainly determined by the climate of the islands. *Agropyron yezoense*, *A. gmelinii* and species of *Elymus* are not found in the area of climate where the warm-temperate evergreen forest is grown, but distributed in Hokkaido or also in Honshu inland prefecture area such as Nagano, which is relatively cool and dry through the year (see Nagano Prefecture Flora Compilation Committee 1997). On the other hand, *A. tsukushiense*, *A. ciliare* and *A. humidorum* are mainly in the area of the warm-temperate evergreen, and in Okinawa (Hatsushima 1971; Walker 1976), the paratropical rain forest area by Wolfe (1979). Naturalized species such as *A. repens* are mainly in an open land of Hokkaido and equally in Nagano prefecture (Yokouchi 1983, see Nagano Prefecture Flora Compilation Committee 1997).

1. Early study of *Triticeae* in Japan

Triticeae species did not appear in publications by pioneer plant collectors in Japan; C. P. Thunberg (1973-1828) came in 1775 and P. F. von Siebold (1796-1866) first came to Japan in 1823 (see Ohwi 1984). The appearance of *Triticeae* species treated by the system of Linnaean nomenclature dates back only to the years of the later nineteenth century, near 1900.

The earliest description that was on page 33 of a publication edited by Matsumura J (1905) is shown in Fig. 1. Besides two *Agropyrum* (*Agropyron*) species, those of the genus, *Elymus*, *Hystrix* identified as endemic plants, are described.

Gramineæ.

(*Kwahon no tagui.*)

Agropyrum caninum, Beauv.; Hack. B. H. B. (1899) 714.

syn. *Triticum caninum*, Schreb.; A. Gr. Perr. Exped. 328; Miq. P. 175; F. S. II. 185.

Nom. Jap.

Hab. Yezo: Sapporo (Isikari), Nagatoyo (Siribesi); Nippon: Gongensaki (Mutsu); Matsusima (Rikzen), Simoda (Idsu); Tsusima.

Agropyrum semicostatum, Nees; Steud. Syn. 346; Hack. B. H. B. (1899) 714.

syn. *Triticum semicostatum*, Miq. P. 175; F. S. II. 185.

Nom. Jap. *Natsu-no-chahikigusa.* ナツノチヤヒキグサ

Kamoji-gusa. カモジグサ

Kazura. カズラ

Hab. Yezo: Kari (Kusiro), Hakodate (Osima); Nippon: Hondo-odji (Uzen), Deyu (Yetsigo), Mt. Adsuma, Wakamatsu (Iwasiro), Matsu sima (Rikzen), Nandjoo (Sinano), Tokio (Musasi), Mt. Amagisan, ins. Hatsidoo (Idsu), Amatsu (Boosiu), Mt. Tsukba (Hitatsi), Harakosai (Bittsiu), Hikami (Suwoo); Sikok: Kusaka (Tosa); Tsusima.

Fig. 1. A photograph showing the earliest description of *Agropyron*.
Two species are described on Page 33 in Matsumura J (1905). Identification of Kamoji-gusa of those days was *A. semicostatum*.

Elymus species were *E. arenarius*, *E. dahuricus* and *E. sibiricus*, and in *Hystrix*, two species were included under the name of *Asprella japonica* and *A. sibirica* var. *longearistata*. Although they are not native, *Hordeum murinum* and *H. sativum* are listed. Presumably *H. murinum* was already among the naturalized and established plants within some decades after Japan opened the country at about the middle of 1800's. Also in the list wheat and three varieties of the cultivated barley, *H. sativum* var. *hexastichon*, var. *vulgare* and var. *distichon*, which except var. *distichon*, came to Japan as crops probably two thousands years ago, are included. For naturalized plant species, I will discuss the cases more on later pages.

Identification of species of *Elymus* and *Hystrix* has not largely changed during last 100 years. However, identification of *A. caninum* was not correct by present-day classification. Species name of *Agropyron* went through complications and resulting confusion still remained in 1990's.

During the period after 1868 to the end of nineteenth century, besides European and American ways of study on scientific names taxonomists must have been thoughtful of those of Japanese local vernacular names and Chinese names. Matsumura J (1895) in his authentic work of this period listed Japanese species with traditional local vernacular and equally by corresponding Chinese name. Although Japanese indigenous *Agropyron* (*Roegneria*) plant was identified as *Brachypodium japonicum* Miq., there were three vernacular names, Natsu-no-chahikigusa, Kamoji-gusa and Kazura, and the Chinese characters to write Kamoji-gusa, as shown in Fig. 2.

539. **Boykinia lycoctonifolia** *Engl.* (Saxifrag.) 虎耳草科
Saxifraga lycoctonifolia Maxim.
Arashigusa. アラシグサ

540. **Brachypodium japonicum** *Miq.* (Gramin.) 禾本科
Natsu-no-chahikigusa. ナツノチヤヒキグサ 鷲麥（敬）
Kamoji-gusa. カモジグサ 鷲觀草（敬荒野謙）
Kazura. カヅラ

541. **Brachypodium silvaticum** *R. et S.*
Yama-kamojigusa. ヤマカモジグサ

542. **Brasenia purpurea** *Casp.* (Nymphaea.) 睡蓮科
Junsai. ジュンサイ 蓴（本）
Nunawa. ヌナハ

Fig. 2. A part of page 51 of Matsumura J (1895), the earliest taxonomy book in Western style describing Kamoji-gusa.
Among listed plants, Kamoji-gusa is No. 540 under the scientific name *Brachypodium japonicum* Mig. Japanese vernacular names together with Chinese word in Kanji (Chinese character) are described.

2. *Triticeae* species seen from the ethnobotanical point of view

In spite of the abundance and general distribution of the *Agropyron* and *Elymus* species, ethno-botanically those wild *Triticeae* species were of little interest to the people inhabited the islands throughout the history. They seldom or never depicted in Japanese traditional pictures, nor described in documents. Clearly, people were indifferent to the plants and neglected use, although some authors who described traditional publications of encyclopedia type included them among other ornamental and horticultural plants and varieties (see Kitamura et al. 1988).

Vernacular name "Kamoji-gusa" is especially given to one species, *A. tsukushiense*, in present day Japan. When translated "Kamoji-gusa" in Japanese to English, a meaning of "Kamoji", is a hair piece, false hair, or a switch. Walker (1976) described *A. tsukushiense*, then Japanese Kamoji-gusa, gave as English "Switch grass" in his book of "Flora of Okinawa and the Southern Ryukyu Islands" as a meaning of Japanese. To this direct and precise translation I am quite agreeable. However, we must carefully consider another plant with the same common name, which is Switch grass (*Panicum virgatum* L.), in the United States (see Hitchcock 1950; Kucera 1961; Steyermark 1981). Nevertheless, it is interesting that both species are commonly found in the areas.

At any rate, *Agropyron* plants are familiar in play ground of children. That is expressed by another old name, Natsu-no-chahikigusa, and surely given rise by the image of tender and linearly shaped leaves and spikes, that is reflected also by the old name of Kazura.

3. Difference between *Agropyron* and *Elymus*

At least the species found in Japan, the genus *Elymus* (excluding *Elymus mollis*) is clearly distinguished from *Agropyron* in spike morphology. Hitchcock (1950) in describing the spike morphology of the genus *Elymus* pointed out that the rachilla distorted at base, bringing the florets more or less dorsiventral to the rachis (Fig. 3). This is characteristic to the genus *Elymus* and probably strongly impressed the late Dr. J. Ohwi. He pointed it out when I met him during the 1950's, and said that the distortion of rachilla is never seen in *Agropyron*, and then he treated it as one of the clear characters to separate the two genera throughout in his works (Ohwi 1941, 1958, 1965, 1983, 1984). In Flora of Japan, which was edited by Meyer FG and Walker EH, the English description is similar to that of Hitchcock (Ohwi 1984). Differences are sharp, in two genera growing under the same conditions. Besides the number of florets is higher in *Agropyron* than in *Elymus* species, these two characters are consistent, while spikelet number per rachis node may vary two to one

Fig. 3. Photograph showing twisted rachilla in *Elymus* spikelets.
Fallen spikelets of *E. virginicus*, an American species, were collected for photo. Cultivated in Japan. At center to right are view from rachis side, and palea (inner glume) is seen because of twisting rachilla. The rest of two are view from the outside.

and sometimes three, in *Elymus*.

4. Naturalized alien species distributed in wild condition of Japan

Naturalized species growing under wild conditions are included in Table 1 (see Note-7). Some species are clearly brought to Japan by human activities after contacting Western countries.

Three categories of the naturalized plant species will be considered. It could be said that there has been some different views in Japan on the sources of species growing on the Japanese archipelagos. Geologically, at least the three islands of Japan, Honshu, Kyushu and Shikoku had been connected to the Asian continent, or, separated depending on the palaeoclimate. The terms such as alien, foreign, naturalized and introduced may be more proper with reality if we are aware that the Japanese islands are separated by sea far enough so that natural distribution of wild species to the islands would not be expected.

Maekawa (1943) discussing on the plant species that are naturalized from one floral district to the different areas brought by human activities, noticed such a group that, at least in Japan, the naturalization by humans not always happen with conscious introduction. In the ancient time before writing a historical documentation, plants might have been brought to Japan in unconsciously along with the main crop species. Thus, the *Triticeae* species now we have in Japan could be divided into four types regarding distribution,

a: pure, endemic and indigenous,
b: prehistoric naturalized plants,
c: accompanied plants with crops,
d: naturalized wild plants (in ordinary sense).

In historical times, it would have been possible that some plants arrived accidentally with the main crops brought to Japan, and they are called accompanied plants. Naturalized plants in ordinary sense are known to include, and for what local descriptions are available are as follows:

A. repens, *Hordeum murinum*, *Ae. cylindrica*, *H. brachyantherum*, *Ae. squarrosa*, *A. intermedium*, etc.

Except the former three species, the rest are thought to be adventitious to a local area such as sea port.

H. murinum is known to be relatively frequent in an area of Tokyo and Yokohama, where there were important main sea ports soon after Japan opened to the Western countries. Also it is thought that plants are able to be settled in areas where nature is disturbed by industrial development. Some recent local publications mention naturalized species in local flora of the prefectures of Nagano (Nagano Prefecture Flora Compilation Committee 1997), Yamagata (Yuhki 1992), and Yokohama City (Yokohama-shokubutsu-kai 2003), etc.

Three *Agropyron* species that are stated to be of foreign origin will be required further detail discussions, whether they are indigenous, or naturalized either prehistoric or as accompanied plants.

(1) Maekawa (1943) listed *A. tsukushiense* var. *transiens*, but it is not included in the lists by Kasahara (1968 etc.). However, Sakamoto (1959, 1961, 1978, 1984) studied an early ecotype of *A. tsukushiense* var. *transiens* and stated that such an accompanied plant line naturalized from China. However, the assumption necessitates further verification.

(2) Later Sakamoto (1978) proposed that *A. humidorum* is also another accompanied species. He observed sympatric distribution and relatively frequent hybridization between *A. humidorum* and an early ecotype of *A. tsukushiense* var. *transiens* (Sakamoto 1966), and assumed that both were naturalized from China. Osada (1989) followed Sakamoto, and stated: "S. Sakamoto says the Japanese plant may have been introduced from China anciently accompanied with *Astragalus sinicus* used as a green manure in the paddy-field". However, the present author does not completely agree with the assumption of being a naturalized plant, rather wishes to reserve a possibility of being an indigenous species (Muramatsu unpub).

(3) Name of *A. caninum* has been reported from 1905. The Japanese name for the species given is now "Ibuki-kamoji" (= switch grass, or, wheat grass of Mt. Ibuki) after the name of Mt. Ibuki (altitude 1,377 m) in Shiga prefecture. Under that name, the plant has been cited frequently without verification in books,

publications and home pages. The species has been interested in Japan, because it has been said to be a remainder of plants introduced to Japan by a missionary accompanied with medical plants in the period when Japan contacted with Western countries and established a herb garden at a base area of Mt. Ibuki in 1500's (before the Tokugawa era) (see Osada 1976; Sakamoto 1982; Satake et al. 1982, etc.). However, the present author wants to argue for necessity of reconsideration and check the specimens. The reason is that the *Agropyron* species in the question differs from the authentic *A. caninum* in Europe by the present author's observation.

The *A. caninum* listed in the publication by Matsumura J was supposed to be comprehensive by present thought, and presumably he had tried to identify scientific name by adopting those found in European countries. In reality, *A. caninum* of that time merely indicated common abundant plants including such species as *A. tsukushiense*, etc. to which taxonomists later gave the new scientific name after 1930's.

IV. The tribe *Triticeae* in Japan; studies after 1930 concerning species and scope of their distribution

1. Classification

Table 1 shows a list of those species described in reports, which treated mainly Japanese floras. Regardless the change of the scientific name used by the authors, the same kind of plant is arranged in the same line and given Japanese vernacular names, which is rather consistently indicating the same kind of plant.

Apparently after an awareness of the distribution of Japanese endemic and indigenous *Triticeae* species, our own nomenclature with new scientific names was started by contemporary Japanese, young taxonomist. Among them Honda (1930) published his study for grasses that Ohwi (1941) praised as Honda's great work (voluminous work), that is *Monographia Poacearum Japonicarum* (1930), with good index and precise and detailed synonyms. During the coming ten years, studies had proceeded beyond Honda. Ohwi reported *Gramina Japonica* I~IV in Acta Phytotaxonomica et Geobotanica from 1941 to 1942. He amended, supplemented Honda's identification and scientific names, and Ohwi himself also amended and changed the name of *Agropyron* species in *Gramina Japonica* I, in the same series of papers. The name of *Agropyron tsukushiense* was changed; the species name *A. Mayebaranum* Honda in 1941 was changed to *A. Kamoji* Ohwi in 1942. Further changes were made and finally the scientific name *A. tsukushiense* Ohwi var. *transiens* Ohwi was given to a plant "Kamoji-gusa". Similar changes of a given species name had made by renaming the present-day *A. humidorum* (Japanese name Mizutakamoji). At first the species was named *A. Mayebaranum* (Honda 1930), and then Ohwi and Sakamoto (1964) named it *A. humidum*. Later Ohwi (1965) corrected to *A. humidorum* in his revised edition of Flora of Japan. The confusion could have been resulted from the relatively frequent hybridization between *A. tsukushiense* var. *transiens* and *A. humidorum*. The present author has observed frequently that the hybrid plants now called "Tariho-no-ohtachikamoji", *A.* x *Mayebaranum*, were more vigorously and conspicuously growing than the parents. And often *A. humidorum* plants were completely absent from nearby areas. Furthermore, since *A. humidorum* shows highly plastic nature that under unfavorable condition on the poor soil such as on the ridge of paddy field, plants become as small as only 15 cm with relatively short spikes or so. For the characteristic propagation aspect of *A. humidorum* in comparison with *A. tsukushiense* see Kimata and Sakamoto (1982). These were probably the reasons that Matsumura S (1941) described a line (2n = 6x = 42, *A. humidorum* at present) as a dwarf species from Kyushu, without having proper identification at the time. Similarly, for *A. ciliare* the scientific name *Agropyron racemiferum* Koiz. is adopted in "New Flora of Japan" of Ohwi, revised by Kitagawa (1983), while *A. ciliare* is used in the English edition published in 1984. In Fig. 4, *A. ciliare* var. *minus* is shown and in Fig. 5, *A. humidorum* and *A. gmelinii* var. *tenuisetum*. *A. humidorum* is often found in humid places and near by water. Fig.6 is a photograph of *A. humidorum* growing at the water's edge.

From field observations widely made for species in Japan, the tendency of variations is considered to be genetic within species and that variation is always very little in the *Elymus* group. It is so high in *A. tsukushiense* that in many cases the plants are different within an area of some square meters; frequently neighboring plants show different characters. This tendency is also observed in *A. ciliare*, though there are fewer variables than in *A. tsukushiense*.

This fact may indicate that the Japanese islands, or at least the Far Eastern area, are a center of variation for both *A. tsukushiense* and *A. ciliare*. This would be a reason why taxonomy had been faced a difficulty to choose a typical plant among these two species, and reflected in an erroneous adoption of species names throughout a period of this 60 years. Therefore, one of the problem in classification has been natural variation of morphology and – accordingly – subdivision of the species; one of this was *A. racemifer* (*A. ciliare*)

Table 1. The tribe Triticeae indigenous and naturalized or found, to grow in Japan (Japanese islands)

Tokugawa era and early Meiji era (Japanese names described in documents etc)	Matsumura J		Honda M	Makino T and K Nemoto		Ohwi, J.
before 1868 and to 1883	1884~1895	1905	1930	1931	1941	1942 (addition & correction)
	Brachypodium	*Agropyrum*	*Agropyron*	*Agropyrum*	*Agropyron*	*Agropyron*
	japonicum Miq..	*semicostatum* Nees	*semicostatum* Nees	*semicostatum* Nees	*Mayebaranum* Honda	*Kamoji* Ohwi
					forma *villosulum* Ohwi	forma *villosum* Ohwi
	a				*tsukushiense* Ohwi	a
			Mayebaranum Honda	*Mayebaranum* Honda		*Mayebaranum* Honda
			ciliare Franchet	*ciliare* Franch.	*ciliare* Franch.	
					forma *Okuyamae* Ohwi	
			var. *submuticum* Honda		forma *submuticum* Ohwi	
			var. *pilosum* Honda		var. *pilosum* Honda	
Natsu-no-chahikigusa			*japonicum* Honda	*japonicum* Honda	var.*Hackelianum* Ohwi	
Kazura			var. *Hackelianum* Honda			
	b				forma *japonense*	
Kamoji-gusa					forma *mite*	b
			yezoense Honda		*yezoense* Honda	
					koryoense Honda	*yezoense* Honda var. *koryoense* Ohwi
					Turczaninovii Drob. var. *tenuisetum* Ohwi	
		caninum Beauv.	*caninum* Beauvois			
	c		*repens* Beauvois	*repens* Beauv.	*repens* Beauv.	
						c
		Elymus	*Elymus*	*Elymus*	*Elymus*	
		dahuricus Turcz.	*dahuricus* Turcaninov	*dahuricus* Turcz	*dahuricus* Turcz.	
			excelsus Turcaninov		var. *excelsus* Roshev.	
					var. *villosulus* Ohwi	
		sibiricus L.	*sibiricus* L.	*sibiricus* L.	*sibiricus* L.	
			yezoensis Honda			
					yubaridakensis Ohwi	
	d	*arenarius* L.	*mollis* Trin.	*mollis* Trin.	*mollis* Trin.	
						d
		Asprella	*Hystrix*	*Asprella*	*Asperella*	
		japonica Hack.	*Hackeli* Honda	*japonica* Hack.	*japonica* Hack.	
		sibirica Trauttv.	*longe-aristata* Honda	*sibirica* Trautv.	*longe-aristata* Ohwi	
	e	var. *longe-aristata* Hack.		var. *longe-aristata* Hack.		e
		Hordeum	*Hodeum*	*Hordeum*	*Hordeum*	*Hordeum*
		murinum L.	*murinum* Linnaeus.	*murinum* L.	*murinum* L.	
				maritimum With		
					brachyantherum Nevski	
						Gussoneanum Parl.

Alleged natural hybrid

Note; 1. Only reports of monograph-type included, omitting brief ones. 2. Japanese name before 1883 are in Matsumura J (1895) etc. 3. Mainly followed the original descriptions without making any correction. 4. *A. caninum* in Matsumura J (1905) may include the different other species by the later taxonomists. 5. Honda (1931) reported *Agropyron semicostatum* var. *viridispica* Honda (Japanese name: Midori-kamoji). 6. In Ohwi (1984) specific epithet is [*humidum*] on page 153. However, *humidorum* is used in the description of the species (page 154). 7. *Agropyron repens* and the species of genera, *Hordeum* and *Aegilops*, are not indigenous to Japan.

(continued)

Ohwi, J.		Koyama, T.	Osada, T.	Japanese name (species)	Proposition Groups of similar species, varieties and formas indicates possible coenospecies	
1972	1984	1987	1989, 1993			
Agropyron	*Agropyron*	*Agropyron*	*Elymus*		*"Tsukushiense"*	
tsukushiense Ohwi var. *transiens* Ohwi	*tsukushiense* Ohwi var. *transiens* Ohwi	*tsukushiense* Ohwi formatransiens T. Koyama forma *villosum* T. Koyama	*tsukushiensis* Honda var. *transiens* Osada	Kamoji-gusa		
a var. *tsukushiense*	var. *tsukushiense*	forma *tsukushiense*	*tsukushiensis* Honda	Ke-kamojigusa Oni-kamojigusa		a
humidorum Ohwi et Sakamoto	*humidorum* Ohwi et Sakamoto	*humidorum* Ohwi et Sakamoto	*humidus* Osada	Mizuta-kamojigusa, O-tachi-kamojigusa	*Humidorum*	
ciliare Franch. var. *minus* Ohwi	*ciliare* Franch. var. *minus* Ohwi	*ciliare* Franch. ssp. *minus* T. Koyama	*racemifer* Tsvel.	Ao-kamojigusa	*"Ciliare"*	
var. *pilosum* Honda	var. *pilosum* Honda	forma *submuticum* T. Koyama		Ke-aokamojigusa Ko-aokamojigusa Usuge-kamojigusa		
b		*ciliare* Franch. ssp. *amurense* T. Koyama	var. *japonensis* Osada	Tachi-kamojigusa Hosoba-kamojigusa Tachi-kamojigusa Me-kamoji		b
yezoense Honda	*yezoense* Honda	*yezoense* Honda	*yezoensis* Osada	Ezo-kamojigusa, Ko-kamojigusa	*"Yezoense"*	
var. *tashiroi* Ohwi	var. *tashiroi* Ohwi	forma *tashiroi* T. Koyama	*yezoensis* Osada var. *koryoensis* Osada	Taishaku-kamojigusa, Koryo-kamojigusa		
gmelinii Scribn. & Smith var. *tenuisetum* Ohwi	*gmelinii* Scribn. & Smith var. *tenuisetum* Ohwi	*gmelinii* Scribn. & Smith ssp. *tenuisetum* T. Koyama	*gmelinii* Tsvel. var. *tenuisetus* Osada	Inu-kamojigusa		
caninum Beauv.	*caninum* Beauv.	*caninum* Beauv.	*caninus* L.	Ibuki-kamojigusa		
repens Beauv.	*repens* Beauv.	*repens* P. Beauvois.	*repens* Gould	Shiba-mugi, Hime-kamojigusa	*Repens*	
c						c
Elymus	*Elymus*	*Elymus*				
dahuricus Turcz.	*dahuricus* Turcz.	*dahuricus* Turcz. ssp.*dahuricus*	*dahuricus* Turcz.	Hama-mugi	*"Dahuricus"*	
var. *villosulus* Ohwi	var. *villosulus* Ohwi	*dahuricus* Turcz. ssp. *villosulus* T. Koyama	*dahuricus* Turcz.var. *villosulus* Ohwi	O-hamamugi Yama-mugi		
sibiricus L.	*sibiricus* L.	*sibiricus* L.	*sibiricus* L.	Ezo-mugi Ainu-komugi	*Sibiricus*	
yubaridakensis Ohwi	*yubaridakensis* Ohwi	*yubaridakensis* Ohwi	*yubaridakensis* Ohwi	Takane-ezo-mugi		
mollis Trin.	*mollis* Trin.	*arenarius* L.. ssp. *mollis* Hulten.	*Leymus mollis* Pilger	Tenkigusa, Kusado, Hama-ninniku	*Mollis*	
d						d
Asperella	*Asperella*	*Asperella*	*Hystrix*			
japonica Hack.	*japonica* Hack.	*japonica* Hack.	*japonica* Ohwi	Iwatakeso	*Longearistata*	
longe-aristata Ohwi	*longe-aristata* Ohwi	*duthiei* O. Stapf.	*longe-aristata* Honda	Azumagaya		
e						e
Hordeum	*Hordeum*	*Hordeum*	*Hordeum*			
	murinum L.	*murinum* L.	*murinum* L.	Mugi-kusa		
			marinum Huds.	Hama-mugikusa, Ko-hamamugi		
			brachyantherum Nevski	Chishima-mugikusa		
hystrix Roth	*hystrix* Roth	*hystrix* Roth	*hystrix* Roth	Hime-mugikusa		
			pusillum Nutt.	Minato-mugikusa		
			jubatum L.	Hosonoge-mugikusa		
			Aegilops			
			cylindrica Host	Tsutsuho-komugi, Yagi-mugi		
Agropyron A. x *mayebaranum* Honda [Between *A.humidorum* and *A. tsukushiense* var. *transiens*]		*Agropyron* A. x *mayebaranum* Honda [Between *A.humidorum* and *A. tsukushiense* var. *transiens*]		Tarihono-o-tachikamoji		
A. x *nakasimae* Ohwi [Between *A. ciliare* var. *minus* and *A. tsukushiense* var. *transiens*]		*A.* x *nakasimae* Ohwi [Between *A. ciliare* var. *minus* and *A. tsukushiense* var. *transiens*]		Zarage-kamoji		

Fig. 4. A good stand of *A. ciliare* var. *minus* on a dry riverbed (Okayama, May, 2001).
Plants grow more than 1 meter in Okayama.

Fig. 5. *A. humidorum* (left) on paddy field ridge in Okayama, and *A. gmelinii* var. *tenuisetum* (right) growing in Nagano Prefecture (Muramatsu 1994).

that was divided into several varieties and formas (Kitagawa 1967).

Since those indigenous species are very variable at least in morphological characters it is evident that we are facing diversification of a species. Consequently,

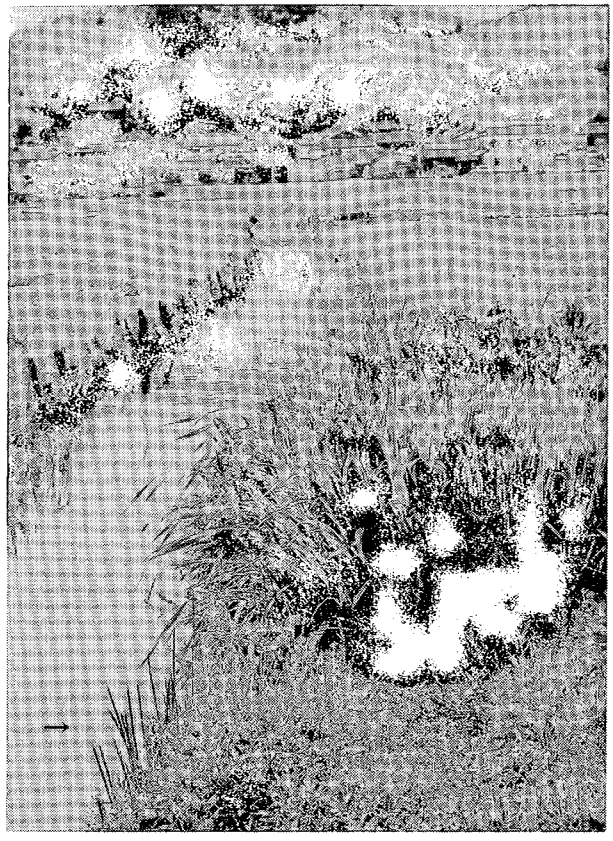

Fig. 6. A photograph of *A. humidorum* growing natural at the water's edge beside Iris (cultivated) (Okayama, May, 2001).
An arrow indicates the plant at heading stage.

the present author proposes those groups to make into one large group as indicated in Table 1. In the Turesson's paper, a sense of genetic evolution is involved and coenospecies is a group of paralogous, sympatric species, which differentiated by evolution (Turesson 1922). *A. ciliare* and *A. tsukushiense* are thought to be differentiated at Japan, at least in the Far East. Subdivided species and varieties are undoubtedly closely related within a large species. The two species, *A. tsukushiense* and *A. ciliare* are each making the group under a sense of "coenospecies". Also I propose that *A. yezoense* and *A. gmelinii* are possible member of a coenospecies, since they hybridize and the F_1 showed normal fertility (Muramatsu 1993).

Sakamoto (1964) tried to classify *Agropyron* species from the basis of cytogenetics, and then wrote a comprehensive paper on patterns of phylogenetic differentiation of the tribe *Triticeae* (Sakamoto (1973). Although in the report wide genera on the worldwide scale were included, the ecological difference among the domestic Japanese species was omitted when he divided the distribution of the *Triticeae* genus into Mediterranean-Central Asiatic regions and Arctic-temperate group. *Agropyron* was included in the latter one. In five genera of the group in *Agropyron* and

Elymus high level of ploidy, from 4x to 12x, is known and natural intergeneric and interspecific hybrid formation is extensive. He further subdivided the group into (1) *Agropyron- Elymus- Sitanion-* complex (2) *Asperella* (*Hystrix*) and (3) *Hordeum*. The *Agropyron-Elymus-Sitanion* complex is divided into five groups. The second group of the complex, which comprised of three genera *Agropyron*, *Elymus* and *Sitanion*, is represented by the European *A. caninum* P. Beauv. Most species of section *Roegneria* of the genus *Agropyron* are included here. The common genome of the group is that of *A. spicatum* Scribn. et Sm. or *A. libanoticum* Hack. Their genome symbol is S by Dewey (1972).

2. From wheat cytogenetics to *Agropyron* study

Genetic study in Japan started when genome analysis of *Triticum* was extended to the closely related genera and then *Agropyron* (see Nishiyama 1954). That was by means of cytological observation and had been carrying out during the period of 1920's to 1940's in Kyoto. As stated previously, since the classification of some indigenous species had not been clear for long, necessity was strongly felt for cooperative study between both field of specialities, cytogenetics and grass classification. This incipient motivation to clear up the indigenous species lasted for many decades up to the present days.

It is clear from Table 1, classification had complications in *A. tsukushiense* and, also *A. ciliare*. Consequently, at least, it had been noticed that the differences of characteristics which were observed among strains of the *A. ciliare* group and those of the *tsukushiense* group, are just of the same degree of difference as seen among the cultivars of wheats and barleys.

3. Early study of cross hybridization and cytological observations

Chromosome studies by 1940 found both hexaploid (6x) and tetraploid (4x) species (Nakajima 1936 and see Matsumura S 1941). Matsumura S (1941) carried out crossing experiments within the genus *Agropyron* and with *Triticum* using *Agropyron* species, either Japanese, or introduced species, which had been received from Canada, *A. elongatum* from K. W. Neatby, and *A. glaucum* from J. M. Armstrong during the later 1930's (Matsumura S 1941, 1948). It was found that hybridization was possible within Japanese indigenous species and also within the section *Elytrigia* and equally between the species of section *Elytrigia* and *Triticum*, and was unsuccessful when Japanese species were tried with the *Elytrigia* species or with *Triticum*.

The F1 hybrid between two Japanese *Agropyron* species, *A. ciliare* x *A. tsukushiense* var. *transiens* (*A. semicostatum* in Matsumura S 1941, 1948), becomes pentaploid (2n=5x=35). The amount of chromosome pairing showed the presence of two common genomes between the parents, just as between the hexaploid and tetraploid wheats. However, different from wheat, the *Agropyron* pentaploid hybrid was completely sterile. Genetic reason for the sterility has not been studied since Matsumura S (1948) first noticed it. In his observation of chromosome pairing in the other two cross combinations regarding *A. tsukushiense* as one parent to *A. humidorum* (2n=6x=42) and *A. cristatum* (2n=2x=14) (Matsumura S 1942, 1948), no good MI cell was obtained in the former hybrid. From the hybrid plant (2n=4x=28) of the latter combination, the amount of bivalent formation was low, ranged from 28' to 1'''+ 3''+18', indicating no common genome present between the parents.

In the cross combinations involving *Agropyron intermedium* (*A. glaucum*, 2n = 6x = 42, introduced from the Voroney* Botanical Garden, USSR) with tetraploid wheats, the amount of chromosome pairing compared between the F1 plants with different genome constitutions, one involved the B genome of wheat (2n = 4x = 28, AABB, *Triticum turgidum*), and the other G genome (2n=4x= 28, AAGG, *T. timopheevi*). Matsumura S (1949) found that pairing was higher in the former hybrids than that in the latter. An average of 7 bivalents in the former hybrid was assumed to be between B genome chromosomes of the parents. However, that assumption was not valid, because in the F1 between an autotetraploid line of *T. aegilopoides* (2n=4x=28, AAAA) as female to *A. intermedium*, the extent of chromosome pairing was higher than at autosyndesis of two homologous A genomes from the female parent (Muramatsu 1955a, b). Besides, also the formation of bivalents, and their variations among cells indicated strongly the existences of genetic system controlling chromosome pairing. Bivalents appeared in the F1 *Aegilops cylindrica* (CCDD) x *A. intermedium* and *Ae. variabilis* (UUS^{1}S^1) x *A. intermedium* with chromosome paring of 5''+ 25'~ 10''+15', and in F1, *Ae. longissima* (S^{1}S^1) x *A. intermedium* chromosome parings were 1'''+5''+15' ~ 10''+ 8' (Matsumura S and Muramatsu 1956; Muramatsu and Sakamoto 1956; Matsumura S, Muramatsu and Sakamoto 1958a, b). Since from these hybrids the B genome is absent, the involvement of B genome is excluded in *Agropyron* (*Elytrigia* group). Then we gave *A. intermedium* a genome symbol EEFFJJ.

Inconsistently, however, the highest number of the associated chromosomes in the F1, *T. durum* 8x (2n=56,

*Presumably Voronezh as pointed out by Dr. G. P. Rédei. Seeds were received through the Kyoto Botanical Garden and the original record is not available at present

Table 2. Main wild *Triticeae* lines used for investigations in 1950's~1960's, and somatic chromosome numbers

Species	2n	Source
A. ciliare var. *minus*	4x=28	Japan
A. gmelinii var. *tenuisetum*	4x=28	Japan
A. yezoense	4x=28	Japan
A. humidorum (A. humidum)	6x=42	Japan
A. tsukushiense var. *transiens*	6x=42	Japan
A. gmelinii Nepal strain	4x=28	Nepal
A. semicostatum	4x=28	Nepal
A. trachycaulum	4x=28	USA
A. repens	6x=42	Japan
A. glaucum (*A. intermedium*)†	6x=42	USSR
A. elongatum	2x=14	Canada
"	10x=70	Canada
E. sibiricus	4x=28	Japan
E. dahuricus	6x=42	Japan
E. mollis (*L. mollis*)	4x=28	Japan
Eremopyrum triticeum	2x=14	Canada
Eremopyrum distans	2x=14	KUSE‡
Eremopyrum buonapartis	4x=28	KUSE
Henrardia persica	2x=14	KUSE
Heteranthelium piliferum	2x=14	KUSE
Taeniatherum asperum	2x=14	KUSE

† Also *A. trichophorum*, ‡ The Kyoto University Scientific Expedition to the Karakoram and Hindukush, 1955 (see Sakamoto and Muramatsu 1965)

AAAABBBB) and *A. trichophorum* (=*intermedium*, 2n=6x=42), was lower than those observed in the hybrids of the cross combinations involving *Aegilops* (Muramatsu 1956). Those results indicated the involvement of the genotype(s) to control the chromosome pairing. Talking with Dr. Okamoto, when I was studying under the late Dr. E. R. Sears on the monosomic series of wheat, the presence of a genetic system was taken into consideration, and together with his result of asynaptic effect of 5B (Okamoto 1957), the idea was supported.

4. Relationships of Japanese species with Nepalese and American species

After the introduction of materials from South-West Asia search of the genome homology by means of cytological studies, extended into the rest of the *Triticeae* genera (Matsumura S and Sakamoto 1955; Matsumura S et al. 1956; Sakamoto and Muramatsu 1962, 1963, 1965). Table 2 is a list of wild *Triticeae* species, which were used for the incipient investigations. However, the investigation was confronted with the barriers in hybridization work. One of the main reasons was the necessity of regulating flowering time to escape the Baiu season, and the second, the inability of endosperm to form mature normal seed. Before overcoming the barrier by embryo rescue, the studies on the cross-compatibility, chromosome pairing and genome analysis became the main subjects followed for decades to the 1980's. To starting with, cross combinations among the same ploidy levels are shown below:

(1) Japanese 4x x Japanese 4x including *A. yezoense*, *A. ciliare* and *A. gmelinii* (Japan),

(2) Japanese 4x x Nepalese 4x including *A. ciliare*, *A. semicostatum*, *A. yezoense* and *A. gmelinii* (Nepal),

(3) Nepalese tetraploid: *A. gmelinii* x *A. semicostatum*,

(4) Japanese 4x x American 4x: *A. ciliare* x *A. trachycaulum*, and

(5) Japanese 6x x Japanese 6x: *A. humidum* x *A. tsukushiense*.*

In the combinations involving *A. gmelinii*, there were problems; pollination was difficult because of Baiu, or a hybrid weakness. However, in the rest of combinations hybrid plant grew vigorous, and the cytological observations are summarized as follows (Table 3) (Sakamoto and Muramatsu 1966a).

(1) Genomes of two Japanese tetraploid species *A. ciliare* and *A. yezoense*, are identical with a mode of 7 bivalents, (2) two hexaploid species, *A. humidum* (*A. humidorum*) and *A. tsukushiense*, have identical genomes, (3) genomes of a Nepalese tetraploid, *A. semicostatum* are closely related to those in Japanese tetraploids, and (4) an American tetraploid species, *A. trachycaulum*, is distantly related to the Japanese tetraploid, showing low bivalent formations ranging from 2 to 9 (with a mode of 5) bivalents. (5) Complete pollen sterility and high seed sterility were the rule in the interspecific hybrids, despite good chromosome pairing at MI.

Investigation between different ploidy interspecific pentaploid hybrids, are shown in Table 4 (Sakamoto and Muramatsu 1966b). A mode of bivalents numbers 14 in the F_1, *A. tsukushiense* x *A. ciliare* supported the result by Matsumura S (1941, 1948). The combinations using *A. yezoense* and *A. gmelinii* (Japan) to *A. tsukushiense* showed essentially similar results. The lower number of bivalents in the tetraploid hybrids between Japanese and Nepalese strains is similarly found in the pentaploid cross combination.

Based on chromosome homology, the genome formulae given at the time were:

Japanese species	2n	Genomes
4x : *A. ciliare, A. yezoense, A. gmelinii*	28	IIKK
6x : *A. tsukushiense* var. *transience*, *A. humidorum*	42	IIKKLL
Nepalese		
4x : *A. semicostatum, A. gmelinii*	28	$I^NI^NK^NK^N$

*Hereafter, the description will be *A. Tsukushiense* instead of *A. tsukushiense* var. *transiens*.

Table 3. Chromosome pairing of the F1 hybrid plants of the cross combinations between the species of the same ploidy (Sakamoto and Muramatsu 1966a)

Cross combination (♀ x ♂)	No. of cells examined	Range of bivalents	Mode of bivalents	Average chromosome pairing				
				V	VI	III	II	I
Japanese 4x – Japanese 4x								
A. yezoense x *A. ciliare*	45	10 - 14	14		0.36	0.20	11.29	2.29
Japanese 4x – Nepalese 4x								
A. ciliare x *A. semicostatum*	408	5 - 14	12		0.14	0.15	11.34	4.20
A. semicostatum x *A. yezoense*	69	10 - 14	12	0.002	0.04	0.04	12.26	3.17
Japanese 4x – American 4x :								
A. ciliare x *A.trachycaulum*	325	2 - 9	5		0.02	0.07	5.33	17.10
Japanese 6x – Japanese 6x :								
A. humidum x *A. tsukushiense*	46	20 - 21	21			0.02	20.74	0.46

Genomic affinity of the Japanese *A. gmelinii* was deduced indirectly from the results, *A. gmelinii* (Japan) x *A. tsukushiense*, *A. tsukushiense* x *A. ciliare* and *A. tsukushiense* x *A. yezoense*.

5. Further study on hybrid and cytogenetic investigations

Evidently the three genomes of *A. tsukushiense* are equally differentiated, because a polyhaploid plant showed low chromosome pairing: the highest number of bivalents per cell was 3"+ 5', in only 0.2% of MI cells, and in 84% bivalent was not observed (Sakamoto 1964a). The amount of bivalent formation at MI is equivalent to a polyhaploid in hexaploid wheat. Backcross experiments yielded, however, 27 plants out of 4,018 flowers pollinated, and then only two were monosomic lower than that expected in wheat. Further cross experiment to explore for the cytological relationships was made with other *Triticeae* genera, *Elymus*, *Heteranthelium*, *Henrardia*, *Eremopyrum* and *Hordeum* (Sakamoto and Muramatsu 1963; Sakamoto 1967a, 1967b, 1968, 1969, 1971, 1972, 1974). Among them genomes of *Heteranthelium* (genome designation at present : Q), *Henrardia* (genome designation : O) and *Eremopyrum* (genome designation : FXe) are highly differentiated showing little homology to the chromosome of the other genera. On the other hand, the relationships of indigenous *Agropyron* to *Elymus* species were important, for they contain common genomes (Sakamoto 1965, 1982). Between two *Elymus* species, *E. sibiricus* (2n=4x=28) and *E. dahuricus* (2n=6x=42), there is only one genome in common regardless of the morphological similarities. By contrast, *E. dahuricus* and *A. tsukushiense* are cytologically very closely related irrespective of their morphological classification into the different genera. The two species share at least two homologous genomes showing 17.67

Table 4. Chromosome pairing of the F1 hybrid plants of the cross combinations between the species of the different ploidy (Sakamoto and Muramatsu 1966b)

Cross combination (♀ x ♂)	No. of cells examined	Range of bivalents	Mode of bivalents	Average chromosome pairing				
				V	IV	III	II	I
Japanese 4x – Japanese 6x								
A. tsukushiense x *A. ciliare*	107	9 - 15	14		0.06	0.08	13.07	8.44
A. tsukushiense x *A. yezoense*	87	7 - 15	14		0.15	0.33	12.24	8.81
A. yezoense x *A. tsukushiense*	55	13 - 15	14	0.04	0.18	0.29	12.60	8.01
A. gmelinii (Japan) x *A. tsukushiense*	50	11 - 15	13		0.18	0.16	12.28	8.84
A. humidum x *A. ciliare*	53	12 - 16	14		0.09	0.02	13.17	6.45
Nepalese 4x– Japanese 6x								
A. tsukushiense x *A. semicostatum*	155	6 - 14	11		0.11	0.16	9.80	14.05
A. humidum x *A. semicostatum*	47	8 - 14	10			0.02	10.66	13.62
A. gmelinii (Nepal) x *A. tsukushiense*	97	3 - 15	12		0.11	0.11	11.30	11.57
A. gmelinii (Nepal) x *A. humidum*	25	9 - 15	11		0.04	0.08	11.92	10.76

bivalents per cell. Sakamoto (1982) concluded that these three species *E. sibiricus*, *E. dahuricus* and *A. tsukushiense* contain one homologous genome in common, and that this genome will be Dewey's S genome (Dewey 1974), which is either I, K or L genome designated for *A. tsukushiense* by Matsumura S (1941, 1948).

Since Dewey (1974) had reported *E. sibiricus* is genomically similar to *A. caninum* and *E. canadensis*, which contained the H genome from *Hordeum* besides the S genome. Thus the genome relationship of the Japanese species to other Asiatic and North-American species became clear, and this was a basis for grouping the Japanese indigenous *Triticeae* species (except *E. mollis*) into the same genetic group II in *Agropyron-Elymus- Sitanion* Complex (Sakamoto 1973).

6. Studies on cross compatibility using Japanese species and cytological observations – chromosome configurations, amphiploidy, polyhaploidy, and anomalous cell-divisions

(1) *Agropyron* - *Triticum* hybrid by embryo rescue

Production of viable hybrids involving Japanese *Agropyron* (*Roegneria*) species with wheats (*Triticum*) was realized by means of embryo rescue. Usually seeds formed by pollinating *Roegneria* species as female with wheat pollen, cease to develop in about a week to ten days, at most 2 weeks after pollination, and then become abortive, shriveled, empty and are unable to germinate.

Takahashi and Muramatsu (1981) by pollinating 2,203 flowers of *A. tsukushiense* as female by pollen of *Triticum aestivum* cv. Chinese Spring obtained 214 (9.7%) seeds. Two F_1 hybrid plants were developed out of 100 embryos cultured, while 70 seeds without embryo culture did not germinate. Hybrids (2n=6x=42; ABDSHY) showed good growth and were morphologically intermediate between the parents. Chromosome pairing was compared between tillers divided from one plant and grew under different conditions. Up to 5 bivalents were observed and 49% of the cells had 42 univalents in tillers kept under favorable condition, 20°C and 12 hrs of light, while under the unfavorable varied conditions of 2°C~ 25°C and 14~24 hrs of light, 95% cells showed 42 univalents. Although there was very low homology between the 6 genomes involved, apparently chromosome pairing is promoted under the former conditions and one ring bivalent was observed, while the rest were rod-shaped weakly paired bivalents.

Similarly, the hybridization with wheat was possible using *A. ciliare* as female. Muramatsu and Kaneta (1982) obtained hybrids, using cultivars of *T. aestivum*, cv. Inayamakomugi and cv. Chikuzen. With *A. tsukushiense* as female, a hybrid plant was obtained by pollination with *T. aestivum* cv. Gabo besides with cv. Chinese Spring. Chromosome pairing of F_1 involving *A. ciliare* showed similar tendencies that were observed in the hybrid involving *A. tsukushiense* in the previous year, but one trivalent association was seen in the combination with *A. ciliare*, although its frequency was very low.

(2) *Agropyron* - *Hordeum* hybrid by embryo rescue, chromosome number instability and colchicine treatment

Embryo rescue was used for producing hybrid between *Agropyron* and *Hordeum bulbosum* (2n=4x=28) (Shigenobu and Sakamoto 1981; Shigenobu-Kishimoto and Sakamoto 1982). In their cross combination, *A. repens* (2n=6x=42) and *A. tsukushiense* as female were pollinated with *H. bulbosum* (4x). A peculiarity appeared in the hybrid plants as there was variation of somatic chromosome numbers. In *A. repens* x *H. bulbosum*, somatic chromosome number in root tips was 2n=34 instead of the expected 2n=35, and spike formation delayed to the fourth year after seedling stage, despite prolific tillering heading was poor and in the root tips 1 or 2 chromosomes were reduced. Chromosome numbers counted at MI showed a range of variations from 34 to 30, and bivalents, which displayed autosyndesis of chromosomes of each parent, varied from 7 to 1. In the hybrid, *A. tsukushiense* x *H. bulbosum* (4x), in addition to the expected 2n=5x=35, PMCs with reduced chromosome number of 2n = 32 were found and bivalents varied from 8 to 2, indicating that almost all of the pairing was by autosyndesis of the *H. bulbosum* chromosomes. However, 8 bivalents per cell and appearance of trivalents and quadrivalent were observed, indicating that some chromosomes from both parents might have contributed to the pairing.

They reported another cross *A. elongatum* (2x) x *H. bulbosum* (4x). One plant obtained had a morphology similar to that of the female parent, but had 2n=15 chromosomes. Apparently *H. bulbosum* chromosomes were eliminated and formation of trivalent indicated trisomic nature of the resulting plant (Sigenobu-Kishimoto and Sakamoto 1984).

Chromosome instability is frequently found in certain cross combinations and/or in the backcross generation plants of F_1, *A. ciliare* x hexaploid wheat (2n = 35), as female pollinated by wheat (2n=42). Kaneta and Muramatsu (1983a, b) reported wide variation of chromosome numbers among PMCs. This was certainly not artifact in making microscopic preparations.

Treating tillers of F_1 plants with colchicine solution, fertile sectors appeared and set some seeds, and of the five amphiploid plants obtained three had the expected chromosome number (2n = 70). From the remaining two, one was 2n=69, and another 2n=66 and in this

Table 5a. Overall results of hybridizations involving *Agropyron ciliare* and *A. tsukushiense* with other species in the *Triticeae*

Female parent	Pollinations			Embryo cultures					
	No.of florets pollinated	No. of seeds	%	No. of cultured	No. of germinated	%	Plants developed: No. of plants	% for No. cultured	% for No. pollinated
A. ciliare	6,358	1,696	26.7	877	316	36.0	92	10.5	1.4
A. tsukushiense	6,655	707	10.6	445	336	75.5	227	51.0	3.4
Total	13,013	2,403	18.5	1,322	652	49.3	319	24.1	2.5

(Cited from Muramatsu et al. 1992, Hereditas offprint Vol. 116, Proceedings of the International TriticeaeSymposium, Helsingborg, Sweden, 1991)

Table 5b. Differences between two *Agropyron* species in the number of successful cross combinations obtained from crosses with other species in the *Triticeae*

Female parent	No. of cross combinations			No. of F1s developed		
	Intergeneric	Interspecific	As line	Genera(%)	Species (%)	Lines(%)
A. ciliare	7	23	48	7(100.0)	16(69.6)	24(50.0)
A. tsukushiense	7	22	45	4(57.1)	6(27.3)	18(40.0)
Total	14	45	93	11(78.9)	22(48.9)	42(45.2)

(Cited from Muramatsu et al. 1992, Hereditas offprint Vol. 116, Proceedings of the International Triticeae Symposium, Helsingborg, Sweden, 1991)

generation chromosome variations were not observed in root tip cells (Kaneta and Muramatsu 1983a).

(3) Further detailed deep investigation on the distant hybridization plants.

During a period of years from the middle of 1980's to the middle 1990's, studies were mainly made concentrating on the following subjects, and the results will be summarized as below:

(a) Cross compatibility of *A. tsukushiense*, *A.ciliare* and *A. humidorum* with the rest of genera in *Triticeae*.

(b) Chromosome number instability and production of polyhaploid plants.

(c) Production of amphiploids.

(d) Analysis of the chromosome pairing configurations using *Agropyron* species.

(e) Production of addition monosomics.

(f) Studies on agronomically useful characteristics in relation to plant genetics and breeding.

(g) Anomalous cell divisions.

(a) Compatibility

Extensive studies were carried out on cross compatibility using *A. tsukushiense* and *A. ciliare* as female, pollinated by species and strains of the genera involving *Triticum*, *Aegilops*, *Elymus*, *Leymus*, *Hystrix* and *Hordeum**. In all cross combinations, it had been tested that seeds obtained are abortive and never germinate. The hybrid plants are only possible to obtain by means of embryo rescue (Kaneta et al. 1984, Muramatsu et al. 1983, Muramatsu et al. 1988, Muramatsu et al. 1989). Therefore, the term "cross-compatibility" in this paper will be used in a meaning of ability that a zygote to develop into adult phase, with/without embryo rescue. It was expected that the rate to produce hybrid plant may vary among cross combinations.

Wide and extensive experiments were carried out. With the total number of pollinations included more than 13,000 florets. The results of the paper submitted to the Proceedings of the 1st International *Triticeae* Symposium, Sweden are in Tables 5a~5e (Muramatsu et al. 1992). The totaled results represented experiments conducted during years from 1988~1990. Separate results will be described in the next section. Both *A. tsukushiense* and *A. ciliare* have shown cross-compatibility with *Triticum*, *Aegilops* and *Hordeum* by embryo rescue (Kudo et al. 1990; Muramatsu et al. 1990; Uno et al. 1990; Muramatsu 1991; Muramatsu et al. 1991). With various species of *Triticum*, *A. ciliare* produced a total of 45 hybrid plants out of 1,982 florets pollinated, while in *A. tsukushiense* two plants out of 1,756 pollinated. Similarly, the difference was clear when *A. ciliare* as female was pollinated by wheat and

*Study was subsidized by a Grant in aid for Scientific Research C from the Ministry of Education, Science, Sport and Culture, Japan.

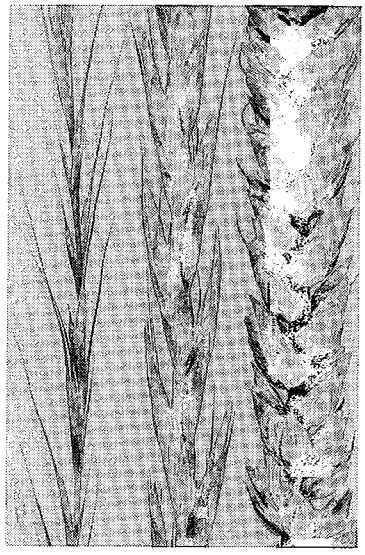

Fig. 7. Spikes of the F1 hybrid plant, *A. humidorum* x *T. aestivum*, and the parents.
A. humidorum (left), F1 (center), *T. aestivum* cv. Chikuzen (right) (Muramatsu et al. 1993).

yielded 18.9 % of seed set, while in *A. tsukushiense* only 0.7%. Also, higher tendencies were obtained with pollen of *Aegilops* species and *Secale*. However, it is interesting that when barley pollen, *H. vulgare* and *H. bulbosum*, were used, the tendencies indicated that the number of resulting plants increased in the crosses using *A. tsukushiense*. Out of 2,407 florets pollinated 544 (22.6%) of seeds were obtained and 368 embryos cultured gave 217 (59.0%) plants. With *A. ciliare*, out of 2,151 florets 41.3% of seeds were obtained and from 354 embryos cultured 7 plants (2.0%) were recovered. Results of studies on the *Agropyron* - barley hybrids will be described in a later section.

Crosses with rye was obtained in combinations, *A. tsukushiense* x rye 2x (Kaneta et al. 1984), and in *A. ciliare* x rye (Kudo et al. 1990). The hybridization was also possible with *A. ciliare* and *A. yezoense* as female to *Hystrix longe-aristata* (Muramatsu 2001). With further experiment using *A. humidorum* as an indigenous *Agropyron* species, similarly we produced hybrid plants. Cytogenetic results obtained in the hybrids resembled those involved *A. tsukushiense* (Muramatsu et al. 1992; Nakatsuji et al. 1992; Muramatsu et al. 1993,)

Results show the wide cross-compatibility throughout the genera of the tribe *Triticeae*. However, there are tendencies that the hybrids show cytological abnormalities in certain cross combination, presumably due to the interaction or complementary effects of the genotypes brought together in the hybrid from the parents. In Fig. 7 is shown the spike of the F1 hybrid between *A. humidorum* x *T. aestivum* and the parents.

(b) Chromosome number instability

One of the conspicuous phenomena would be chromosome number instabilities that appear in certain hybrid combinations. Variations in somatic chromosome number were observed, as described above, in the backcrossed progenies of the hybrids between *A. ciliare* as female crossed with hexaploid wheat or *H. bulbosum*, and either *A. tsukushiense* or *A. repens* as female with *H. bulbosum* (Shigenobu-Kishimoto and Sakamoto 1982; Kaneta and Muramatsu 1983a, b).

Much more extensive chromosome number variation than described previously among cells within a plant was found in the cross combination using barley cultivars, *H. vulgare*. To rule out any artifact in producing the microscopic preparations, the hybrids were reinvestigated in 1988 and the same tendency of the wide chromosome number variation was confirmed (Muramatsu et al. 1989).

Detailed observations made on the hybrid materials in 1988 showed that the chromosome numbers varied very widely from 14 to 41 when *A. ciliare* was used as an *Agropyron* parent crossed to barley. In the leaf blade of the hybrids there were several clear sectors, which showed different degrees of tinctures, strongly suggesting chromosome instability (Muramatsu et al. 1990). In addition, there was clear difference in plant growth, which may not be caused by chromosomal anomalies but may rather constitute genetic hybrid weakness. The hybrid plants involving *H. vulgare* as parent showed strong growth inhibition so that plants remained very small and barely had any spike. On the other hand, nearly normal plant growth was seen in the combination with *H. irregulare*.

Further investigation was carried out in the hybrid involving *A. tsukushiense*. As shown in Table 6, of 32 hybrid plants obtained and investigated, all of the plants showed somatic chromosome instability. Cells with somatic chromosome numbers from 14 to 56 were observed in *A. tsukushiense* x *H. vulgare*, and from 22 to 53 in the hybrids involving *H. irregulare* as barley parent. In both combinations the expected numbers of 2n=4x=28 were observed in 63.9% of cells in the former hybrid and in the latter 62.2%. Spike morphology showed big differences among tillers: spikelet per node showed the character of *Agropyron* to barley type that is 3 per node. It is important to note that an individual with 2n=21 cells frequently had spike morphology resembling the *Agropyron* parent (Kudo et al. 1990; Uno et al. 1990).

When the fluctuation leads toward the reduction of chromosome numbers, and if the entire barley chromosomes were eliminated, polyhaploid of the *Agropyron* could be produced. Such tillers or individual plants were formed during the development of the hybrid plants. The elimination process was relatively gradual in the combination of *Agropyron* line "Nakayamashouten". In Fig. 8, a spike of such a

Table 5c. Results of crossing *A. ciliare* (2n=4x=28) and *A. tsukushiense* (2n=6x=42) as female to *Triticum* species and barley chromosome addition lines

Pollen parent		*A. ciliare*					*A. tsukushiense*				
		Pollinations		Embryo cultures			Pollinations		Embryo cultures		
Species	Variety or cultivar	No. of florets	% seeds obtained	No. of cultured	% germinated	No. of plants (%)	No. of florets	% seeds obtained	No. of cultured	% germinated	No. of plants (%)
T. boeoticum Boiss.		190	18.9	10	40.0	4(40.0	)178	0.0	-	-	-
T. monococcum L.		182	19.2	23	34.8	5(21.7)	220	0.0	-	-	-
T. urartu Thumanian		43	0.0	-	-	-	-	-	-	-	-
T. thaoudar Revt.		52	15.4	6	33.3	2(33.3)	62	0.0	-	-	
Sum for 2x wheat		467	16.9	39	35.9	11(28.2)	460	0.0	-	-	-
T. boeoticum 4x		191	13.1	14	64.3	6(42.9)	254	0.0	-	-	-
T. dicoccoides Körn var. *spontaneonigrum*		-	-	-	-	-	39	0.0	-	-	-
T. dicoccum Schrank. 'vernal emmer'		100	28.0	21	9.5	0	263	0.8	2	0.0	-
T. dicoccum var. *atratum*		-	-	-	-	-	94	0.0	-	-	-
T. durum Desf. var. *melanopus*		128	28.9	36	16.7	3(8.3)	118	0.0	-	-	-
T. durum var. *hordeiforme*		-	-	-	-	-	45	4.4	0	-	-
T. durum 'Stewart'		100	14.0	12	50.0	1(8.3)	-	-	-	-	-
T. polonicum L. var. *vestitum*		100	12.0	6	16.7	0	114	0.0	-	-	-
T. polonicum var. *gracile*		135	11.9	16	0.0	-	-	-	-	-	-
Sum for 4x wheat		563	19.0	91	16.5	10(11.0)	673	0.6	2	0.0	-
T. aestivum L. 'Thatcher'		76	26.3	20	30.0	2(10.0)	51	0.0	-	-	-
T. aestivum 'Sonora'		210	25.7	52	36.5	9(17.3)	32	6.3	2	0.0	-
T. aestivum 'Ethiopia line'		14	42.9	6	100.0	2(33.3)	132	2.3	3	33.0	1(33.3)
T. aestivum var. *erythroleucon*		120	13.3	16	56.3	0	46	2.2	1	0.0	-
T. aestivum 'Chikuzen'		204	24.0	35	25.7	6(17.0)	-	-	-	-	-
T. aestivum 'Chinese Spring'		138	14.5	20	40.0	5(25.0)	108	1.9	2	100.0	1(50.0)
Sum for 6x wheat		762	21.7	149	38.3	24(16.1)	369	2.2	8	37.5	2(25.0)
Total wheat		1,983	18.9	293	32.4	45(15.4)	1,756	0.7	10	30.0	2(20.0)
C. S. - barley chromosomes †		722	13.7	91	36.3	15(16.5)	518	0.2	1	0.0	

†Addition and substitution lines of barley chromosomes in cv. 'Chinese Spring' of *T. aestivum* L.

(Cited from Muramatsu et al. 1992, Hereditas offprint Vol. 116, Proceedings of the International Triticeae Symposium, Helsingborg, Sweden, 1991)

(Misprints in the original paper are corrected)

Table 5d. Results of crossing *A. ciliare* (2n=4x=28) and *A. tsukushiense* (2n=6x=42) as female to species of *Aegilops, Haynaldia, Secale,* and amphiploid lines

Pollen parent	*A. ciliare*					*A. tsukushiense*				
	Pollinations		Embryo cultures			Pollinations		Embryo cultures		
Variety† or cultivar	No. of florets	% seeds obtained	No. of cultured	% germinated	No. of plants	No. of florets	% seeds obtained	No. of cultured	% germinated	No. of plants(%)
Ae. squarrosa P-24	84	16.7	3	66.7	1(33.3)	120	0.0	-	-	-
Ae. squarrosa P-26	98	13.3	4	0.0	-	152	0.0	-	-	-
Ae. squarrosa P-76	99	22.2	10	0.0	-	-	-	-	-	-
Ae. squarrosa P-93	78	46.2	9	0.0	-	82	0.0	-	-	-
Sum for *A. squarrosa* 2x	359	23.7	26	7.7	1(3.8)	354	0.0	-	-	-
Ae. squarrosa No. 2, 4x	57	1.8	1	0.0	-	103	0.0	-	-	-
Ae. bicornis (Forsak.) Jaub. & Spanch.	106	0.0	-	-	-	94	0.0	-	-	-
Ae. sharonensis Eig	66	0.0	-	-	-	324	0.3	1	0.0	-
Ae. uniaristata Vis.	221	7.7	10	50.0	3(30.0)	441	0.2	1	100.0	0
Ae. ventricosa Tausch. Var. *comosa*	24	0.0	-	-	-	-	-	-	-	-
Ae. columnaris Zhuk.	-	-	-	-	-	16	2.5	2	50.0	1(50.0)
Ae. triaristata Willd. 6x	92	2.2	1	100.0	1(100.0)	54	0.0	-	-	-
Total *Aegilops*	925	11.4	38	21.1	5(13.2)	1386	0.4	4	50.0	1(25.0)
H. villosa (L.) Schur.	48	91.7	26	19.2	2(7.7)	92	2.6	28	60.7	0
S. cereale 'Haru-ichiban'	58	93.1	42	38.1	10(23.8)	56	1.1	16	6.3	1(6.3)
S. cereale 'Petkus' 4x	101	89.1	13	15.4	0	82	4.4	17	47.1	6(35.3)
Total *Secale*	159	90.6	55	32.7	10(18.2)	138	1.2	33	27.7	7(21.7)
Triticale P-43 ‡	109	21.1	8	50.0	3	-	-	-	-	-
Triticale P-164 §	56	19.6	10	60.0	4(40.0)	87	1.1	0	-	-
S^1sS^1sAA P-96 ¶	205	2.4	2	50.0	1(50.0)	271	0.7	1	0.0	-

†Numeral figures are accession and maintenance Nos., ‡ Hexaploid, § Octoploid, ¶ Amphiploid between *Ae. sharonensis* and *T. boeoticum*
(Cited from Muramatsu et al. 1992, Hereditas offprint Vol. 116, Proceedings of the International Triticeae Symposium Helsingborg, Sweden, 1991)
(Misprints in the original paper are corrected)

Table 5e. Results of crossing *A. ciliare* (2n=4x=28) and *A. tsukushiense* (2n=6x=42) as female to *Hordeum* species

Pollen parent		*A. ciliare*					*A. tsukushiense*				
		Pollinations		Embryo cultures			Pollinations		Embryo cultures		
Species	Cultivar	No. of florets	% seeds obtained	No. of cultured	% germinated	No. of plants (%)	No. of florets	% seeds obtained	No. of cultured	% germinated	No. of plants (%)
H. vulgare											
Two-rowed, introduced †											
Two-rowed,	Amsel	137	21.9	18	50.0	0	110	56.4	42	97.6	29(69.0)
Two-rowed,	Kirin-chokuichigo	192	28.6	26	50.0	0	104	12.5	10	50.0	4(40.0)
Two-rowed,	Goshu-chevalier	98	78.6	6	33.3	0	278	29.9	49	81.6	31(63.3)
Two-rowed,	Svanhals	199	35.7	34	76.5	0	219	12.3	25	96.0	9(36.0)
Improved											
Two-rowed,	Ko-A	298	45.6	81	27.2	0	91	2.2	2	100.0	0
Two-rowed,	Seijyo-1	84	32.1	1	100.0	0	94	36.2	16	100.0	10(62.5)
Two-rowed,	Satsuki-nijyo	314	54.1	37	21.6	1(2.7)	282	33.0	61	98.4	53(86.9)
Two-rowed,	Nitta-nijoichigo	108	44.4	9	0.0	-	124	12.9	12	75.5	8(66.7)
Six-rowed, introduced											
Six-rowed,	Gem	66	59.1	29	58.6	4(13.8)	242	12.0	26	50.0	11(42.3)
Six-rowed,	Mokusekko-3	125	23.2	-	-	-	96	25.0	17	100.0	15(88.2)
Land race											
Six-rowed,	Sumiremochi	197	46.2	33	51.5	0	318	26.1	53	86.7	35(66.0)
Linkage tester				-							
Six-rowed,	Col-orange	32	0.0	-		-	18	22.2	4	100.0	2(50.0)
Sum for *H. vulgare*		1,850	41.8	274	42.0	5(1.8)	1,976	23.8	317	87.4	207(65.3)
H. irregulare		102	44.1	21	52.4	0	124	12.1	8	100.0	7(87.5)
H. spontaneum nigrum		81	38.3	29	62.1	1(3.4)	78	28.2	18	61.1	0
H. bulbosum 4x		118	33.9	30	6.7	1(3.3)	229	16.2	25	36.0	3(12.0)
Total *Hordeum*		2,151	41.3	354	41.2	7(2.0)	2,407	22.6	368	82.9	217(59.0)

† Introduced variety, European origin

(Cited from Muramatsu et al. 1992, Hereditas offprint Vol. 116, Proceedings of the International Triticeae Symposium, Helsingborg, Sweden, 1991)

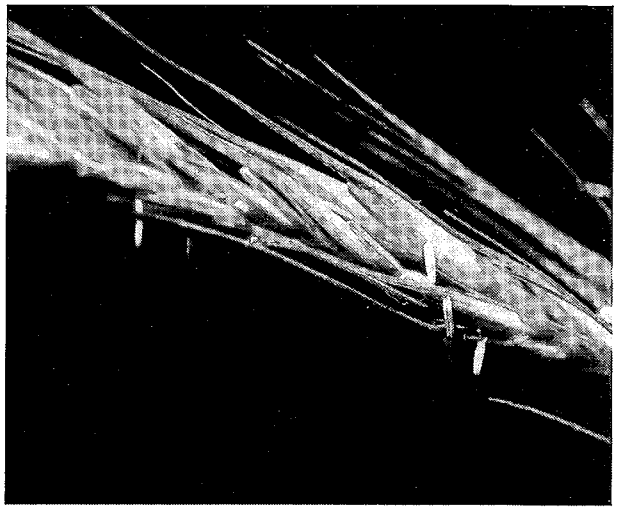

Fig. 8. A part of spike of a polyhaploid-*A. tsukushiense* showing sterility (2n=3x=21).
The plant resulted from the cross, *A. tsukushiense* ♀ x *H. vulgare* ♂. Because of haploidy anthers did not shed pollen, and the plant was sterile.

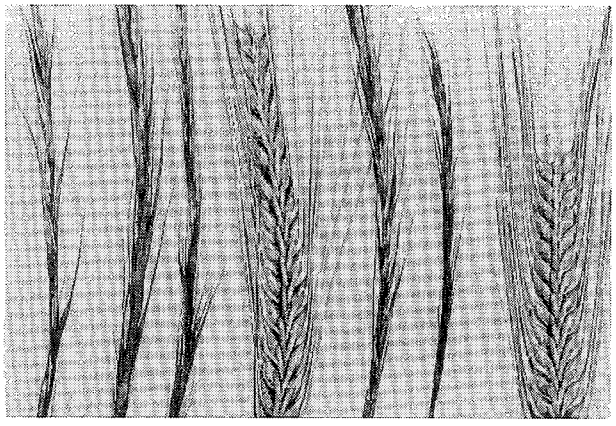

Fig. 9. Results of hybridization, *A. humidorum* ♀ x *H. vulgare* ♂.
At the left, a spike of *A. humidorum*, and the next two spikes are those of the F1 hybrids. The fourth spike from the left is *H. vulgare* cv. Goshu-chevalier. From the fifth spike to right, F1, *A. humidorum* x *H. vulgare* Seijyo 1 with 2n=28 at mode, and 2n=24, and cv. Seijyo 1 (Muramatsu et al. 1993).

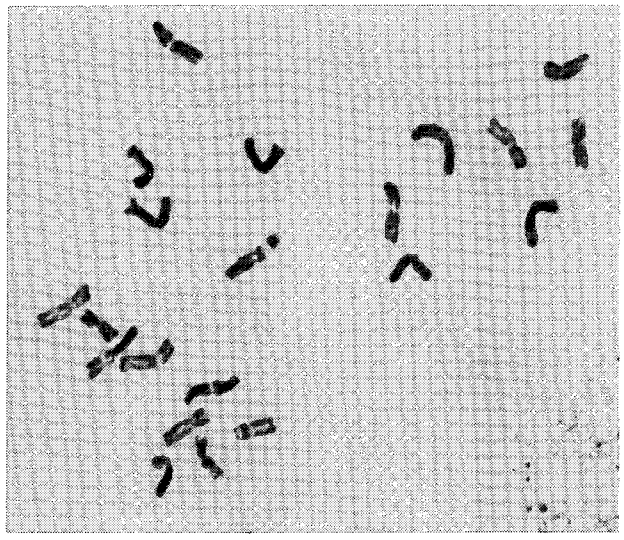

Fig. 10. Somatic chromosomes of a polyhaploid-*A. humidorum* plant (2n=3x=21) resulted from the cross combination, *A. humidorum* ♀ x *H. vulgare* ♂. (Muramatsu et al. 1993).

polyhaploid plant (2n=3x=21) of *A. tsukushiense* is shown. The same phenomenon of the barley chromosomes was observed in the different *A. tsukushiense* line, and also when *A. humidorum* was used as female parent. The elimination seemed to be the same with that in the *A. tsukushiense* hybrids, and resulted polyhaploid plants of *A. humidorum* (Muramatsu et al. 1992, 1993; Nakatsuji et al. 1992). Hybrid plants with the reduction of barley chromosome are shown in Fig. 9, and somatic chromosomes in root tips of a polyhaploid-*A. humidorum* (2n=3x=21) are shown in Fig. 10.

(c) Successful production of amphiploid in relation to the genetic differences

Throughout the investigations, artificial induction of higher auto- and allo- polyploid were tried many times. Whenever it was possible the hybrid plants were treated with a colchicine solution to produce chromosome doubling. With the same treatment given and repeated for years results were clearly different among the cross combinations. In one of the studies to compare the effect of the colchicine between different cross combinations, seven different cross combinations – where the hybrid

Table 6. Instability of somatic chromosome number in the F1 plants obtained by the cross, *Agropyron tsukushiense* var. *transiens* (2n=6x=42), as female x *Hordeum vulgare*, or *H. irregulare*. Expected chromosome number is F1=2n=4x=28 (Uno, Kudo and Muramatsu 1990)

Pollen parents	No. of F1 plants	No. showed chromosome instability	Range of 2n chromosome number	2n chromosome number most frequently observed	% for total cells observed
H. vulgare	24	24	14-56	28	63.9
H. irregulare	8	8	22-53	28	62.2

plants obtained involved embryo rescue or not – were compared (Muramatsu 1985*, Uno and Muramatsu 1989). As shown in Table 7, fertile sector never appeared in the F1 plants of *A. tsukushiense* x 6x-wheat and *A. tsukushiense* x rye, despite the treated tillers clearly showed strong effect of the colchicine solution. On the other hand, the rest of the hybrids effectively formed fertile sectors. The method used was either that of Sears (1941), or of Winkle and Kimber (1976). According to the present author's experience – if properly treated – there is no large difference between the methods of treatment at least in obtaining an amphiploid line, e. g. in the Table 7 F1, *T. spelta* x *A. intermedium*, was by the latter method (Muramatsu and Sigunga 1983).

Clearly, the successful inductions were limited in certain combinations. With repeated experiments, I have never obtained induced octaploid (8x) strain in *A. ciliare* (2n=4x=28, allotetraploid), while octaploid lines (2n=8x=56=AAAABBBB) have been produced in the tetraploid wheats. The author would like to state here a thought of genotypic control for polyploidization; that this difference was throughout the later experiments consistently confirmed. Therefore, I should like to suggest the idea that there is apparently a biological condition for the persistence of certain ploidy. Presumably, genotypic control is involved, that is to say that certain level of amphiploidy is only maintained with a kind of genotype for that ploidy. If we think of that condition, we may have to find a term to designate it, and the author proposes that such kinds of genotypic properties be called "definitomodis genetic conditions"**. Only under that determination of range or level of zone multiplication of chromosome sets will be possible, and a fit ploidy will be realized.

(d) Chromosome pairing configurations of high autopolyploids

Table 7. Result of the colchicine treatment of the F1 hybrid involving *Triticum* and *Agropyron* (Muramatsu 1985)

Cross combination	Result of the treatment
A. trichophorum x *T. dicoccum*	+
T. spelta x *A. intermedium*	+
A. ciliare x 4x-wheat	+
A. ciliare x 6x-wheat	+
A. tsukushiense x 6x-wheat	–
A. tsukushiense x *rye*	–
6x-wheat x rye	+

A. elongatum (2n=10x=70), despite its high polyploidy had been producing seed set in a peculiar manner. Matsumura S (1941, 1948) had concluded that the species is amphiploid. However, the higher chromosome association had not been completely analyzed. Then with suggestion of Matsumura S, the present author with a given clone studied the chromosome mechanics in detail.

A. elongatum showed a regular process of meiotic division, on the other hand showed highly complicated chromosome associations difficult to analysis and also high associations might have been caused by chromosome rearrangement. Nevertheless, the result showed that the species (then the clone) is autodecaploid forming chromosome association up to decavalents (Muramatsu 1990). The important point is that there must be certain proper chromosome arrangement before starting homologous pairing and then chiasmata formation. Choosing partner chromosome ends may not be random. With regard to this non-randomness, the Rabl orientation (see Fussell 1987, i e. the configuration and position of the chromosomes within the nucleus during the cell cycle) would be one of the important factors.

Further test and evidence for the decaploid nature were made and confirmed by observing chromosome associations at MI of a F1 hybrid of the cross combination, an autotetraploid line of *Ae. squarrosa* (2n=4x=28, DDDD) as female to the decaploid *A. elongatum*. The result completely supported autodecaploidy, because up to quinquevalent formation was observed (Muramatsu 1984, unpub).

In observation of the chromosome associations in the hybrid materials involving Japanese indigenous species, evidently a genetic system controlling chromosome pairing is not receiving from or not producing adequate effects on each other. Presumably, the systems controlling pairing may not be very similar in the component genomes. Chromosome association at MI of polyhaploid plant of the *A. tsukushiense* is very similar to the amount of bivalents shown in hexaploid wheat. Slightly higher association was observed in the F1, which seen under favorable growth condition (Takahashi and Muramatsu 1981), is surely by higher number of homoeologous chromosomes involved in the hybrid; six genomes are involved instead of three in each single, polyhaploid of the parent species. In knowing chromosome pairing since prophase association should be important, a microscopic observation was made in the F1 *A. tsukushiense* x 6x wheat. The result was a general tight association at a stage apparently, pachytene, although unpaired single

* Table 1 in the report, *Ae. cylindrica* x *A. dicoccum* should read *Ae. cylindrica* x *A. elongatum*

**Using a Latin term, definitomodis, as one of the suitable words was suggested by Dr. G. P. Rédei

chromosome regions were also observed. This indicated that the high number of univalents of the F1 is due to desynapsis or to lack of chiasma formation (Muramatsu 1982).

(e) Production of alien addition lines

Since the same system of the pairing control is involved, alien addition lines will be expected accordingly. Utilizing the amphiploid and backcrossed generations originated from the cross, *A. ciliare* x hexaploid wheat backcrossed to the wheat parent as female, the chromosome assortment was quite normal and showed random distribution to progeny. *Ciliare* chromosome addition lines were expected to be successful in the later generations. Production projects were carried out by early 1990's (Muramatsu and Ohasa 1992; Takata and Muramatsu 1992; Muramatsu and Takata 1993).

B_2F_1 (the generation back crossed twice) plants of crosses of an amphiploid (2n=10x =70) derived by colchicine treatment of plants, *A. ciliare* "Hyakkengawa" strain x *T. aestivum* cv. Inayamakomugi, were backcrossed to the wheat parent twice. A higher rate for disomic addition plants was obtained in progeny of parent plants with several *ciliare* chromosomes than plants with single chromosome addition. So far, 22 disomic lines were selected and examined for morphological differences and for the flowering date and seed fertility.

(f) Study on agronomically useful characteristics

As stated previously in this paper, Japanese islands are seasonally under "Baiu" during a period of June-July, heavy raining season, which produces conditions with long lasting high humidity for days and weeks under relatively high temperature. Under the condition, fungus infection, especially of scab (*Fusarium* scab , *Fusarium graminearum, or Fusarium sp.*), became prolific on the spikes and grains to various degrees depending on lines. Also, powdery mildew (*Erysiphe graminis*) and leaf rust (*Puccinia recondita*) are found among susceptible cultivars.

In studying reactions of the hybrids to inoculation by powdery mildew spores, Muramatsu et al. (1983) considered the differentiation into forma specialis of powdery mildew fungus, t2 for wheat and a2 for the Japanese indigenous *Agropyron*. Every F1 hybrid plants were tested with lines of forma specialis, kindly supplied from Dr. U. Hiura, Research Institute for Bioresources, Okayama University. Of two cross combinations, *A. tsukushiense* x *hexaploid* wheat, Chinese Spring and Inayamakomugi, the hybrid plants did not get infected by the a2 spores, although both parent species, *Agropyron* and hexaploid wheat cultivars are susceptible to each forma specialis fungus spores, respectively.

On the other hand, infections occurred with t2 spores that represent a forma specialis of wheat. However, in the backcrossed generation of the F1, *A. ciliare* x hexaploid wheat cv. Inayamakomugi, pollinated by the wheat parent, the infection in slight degree was observed probably indicating chromosome instability, which eliminated chromosome(s) carrying gene (or genes).

With the cross using *A. elongatum* and hexaploid wheat non infection by t2 strain, with one exception, was observed (Muramatsu and Ikeda 1983).

Many of the Western cultivars of wheat and barley from dry summer areas are severely infected by scab. Since Japanese lines of *Triticeae* particularly *A. tsukushiense, A. humidorum* and *A. ciliare* show adaptation, tolerance to the Baiu, they will be suitable lines to study these attributes. I have even never seen any single infection of scab on these species for many decades of years.

For the reaction to scab fungus *Fusarium* scab field observations were made whenever the hybrids material was available in June to July, and tendencies were found for the relative tolerance of the hybrids as compared with wheat and barley cultivars in general. However, in addition to the highly variable field conditions, the sterility of the hybrid plants made results not certain for obtaining any conclusive result. Once in a while, weak infection of glumes thought to be caused by *Fusarium* scab were found but the degree of such spots showed fluctuations indicating that further detailed and quantitative studies are required.

Summer survival of the hybrid plants were interesting under conditions of high humidity and high air temperature that continued throughout day and night. The result of the observations summarized by the 1984 season (Muramatsu 1985) is not conclusive but tendencies are that hybrids with *A. elongatum* and *A. intermedium* (both are classified *Tinopyrum* in the different classification system) tolerated the summer season through at least 3 years, while wheat-rye F1 hybrids, as expected, showed completely annual life form. The hybrids involving Japanese indigenous species *A. tsukushiense, A. ciliare* and *A. yezoense* said to display phenotypes ranging from intermediate of the parents to the annual; only a small number of tillers from the hybrid plants, *A. tsukushiense* x hexaploid wheat, showed summer survival at most 3 years.

In addition to the indigenous species, some of Western *Triticeae* lines, if tolerated will be useful. At least two *Agropyron* (*Tinopyrum*) clones, which had been introduced by Matsumura S in 1930's, have survived vegetatively, during a period of almost 70 years. These *Tinopyrum* lines were used as the parents for observed hybrid plants.

(g) Chromosome behavior at cell division not

showing regular distribution or random assortment, rather anomalous divisions are prevalent.

In addition to the description made above, from cytological observations on hybrid plants, there are various kinds of irregularities and disturbances in cell division, depending on the cross combinations. The occurrence of anomalous phenomena that are in connection with the preceding descriptions is thought to be important to summarize here. When *A. tsukushiense* and *A. ciliare* were hybridized to *A. intermedium*, the spikes of the hybrids were perennial to tolerate summer conditions and showed vigorous growth. At meiotic cell divisions showed strong abnormalities, including abnormal spindle fiber formation, and lacking concomitant cell divisions. The abnormality may be due to disturbances of cytokinesis rather than caused by chromosome inhomology. The plants were sterile. Fertile sector was not obtained by colchicine treatment (Muramatsu 1992). Similar, but much more extensive anomalous phenomena were observed at MI of the hybrid, *A. ciliare* x *Hystrix longearistata* (Muramatsu 2001). Also, the author experienced that in another cross combination similar effects of the genotype has been cytogenetically segregating frequently in the progeny of the cross hexaploid wheat and *A. elongatum*. At least, some amphiploids or autoploids have been obtained in the lines where cell division and chromosome behavior were not disturbed by cytokinesis failure.

Conclusions

Genome symbols of *Agropyron* species (conventional classification) indigenous to Japan are at present known to be SH for tetraploidy and SHY for hexaploidy. The genomes are common to *Elymus* (in the narrow sense) species regardless of morphological distinction between genera. In a detailed study on the cross compatibilities, which was wide-ranging, aided by embryo-rescue, throughout the *Triticeae* species available at hand in Japan, a tendency was revealed that the genotype the primary factor in the production of the hybrids that finally develop into mature hybrid plants. This genotype, which is the difference reflecting the phylogenetic groups (genera etc.), may play barrier to separate each one from another. We went deep into the investigation of the anomalous phenomena, often observed cytologically. It could be thought that there are genotypes, which produce chromosome inability or another to keep cells under a stable condition; there are genotypes causing chromosome elimination and irregular cytokinesis. The fact that possible amphiploid formation or the other way making limitation to polyploidization higher than a certain level may be an effect of genotype or genotypes concerning to the factors producing these phenomena. Author, therefore, proposed that we must introduce an idea for ploidy formation, which is a genotype conditioning polyploidy that may be called "definitomodis genetic conditions". Details of this and equally the factors, which produce complete sterility despite of high chromosome pairing that occur in some interspecific hybrids requires further deep investigations.

From ecological point of view, three Japanese *Agropyron*, *A. tsukushiense*, *A. humidorum* and *A. ciliare*, species show unique adaptation and tolerance to the unfavorable summer conditions with long lasting rainy season of Japan, called "Baiu". The species, then, genotypes or genes, determining tolerance conditions, will be fundamental future genetic resources both for theoretical and applied research.

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References

Dewey DR (1972) Cytogenetics of tetraploid *Elymus cinereus*, *E. triticoides*, *E. multicaulis*, *E.karataviensis*, and their F1 hybrids. Bot Gaz 133: 51-57.

Dewey DR (1974) Cytogenetics of *Elymus sibiricus* and its hybrids with *Agropyron tauri*, *Elymus canadensis*, and *Agropyron caninum*. Bot Gaz 135: 80-87.

Fussell CP (1987) The Rabl Orientaion: A prelude to synapsis. In: Moens BP (ed) Meiosis. Academic Press, Orlando, Florida. 275-299.

Hatsushima S (1971) Flora of the Ryukyus. Including Amami Islands, Okinawa Islands, and Okinawa-seibutsu-kyoiku-kenkyukai, Naha (Okinawa) : 1-940 (in Japanese).

Hitchcock AS (1950) Manual of the Grasses of the United States. United States Department of Agriculture Miscellaneous Publication N 200. United States Government Printing Office, Washington DC : 1-1051.
Honda M (1930) Monographia Poacearum Japonicarum, Bambusoideis exclusis. J Fac Sci, Imp Univ Tokyo, Sect III Bot Vol III. part I: 1-484.
Iwateken shokubutsu-no-kai (Botanical Club of Iwate) (1970) Iwateken shokubutsushi (Flora of Iwate) : 1-703 (in Japanese).
Kaneta S and Muramatsu M (1983a) Intergeneric hybrids between the species indigenous to Japan and hexaploid wheat and their progeny generation. 3. Cytogenetic studies of the BF1 generation, F1 (*A. ciliare* x hexaploid wheat) x hexaploid wheat, and amphiploid plants obtained with colchicine treatment. Japan J Breed 33 (Suppl 1): 164-165 (in Japanese).
Kaneta S and Muramatsu M (1983b) Intergeneric hybrids between the species indigenous to Japan and hexaploid wheat, and their progeny generation. 4. Morphological and cytogenetical studies of BF1 generation from F1 [(2n=28) x hexaploid wheat (2n=42)] x hexaploid wheat, particularly on variation of chromosome number within a plant. Japan J Breed 33 (Suppl 2): 60-61 (in Japanese).
Kaneta S, Yoshimura Y and Muramatsu M (1984) Intergeneric hybrids of Japanese indigenous species with wheat and rye, and cytogenetics of the hybrids. Japan J Breed 34 (Suppl 1): 78-79 (in Japanese).
Kasahara Y (1968) Weed of Japan, seeds, seedlings and plants. Yokendo Ltd, Tokyo: 1-517 (in Japanese).
Kimata M and Sakamoto S (1982) Interrelationships between the mode of reproduction and the habitat of two weedy *Agropyron* species, *A. tsukushiense* and *A. humidorum*, Gramineae. Weed Res (Japan) 27: 103-111.
Kira T (1991) Forest ecosystems of East and Southeast Asia in a global perspective. Ecol Res 6: 185-200.
Kitagawa M (1967) Review on the relatives of *Roegneria ciliaris* (Trinius) Nevski. J Jap Bot 42: 219-223.
Kitamura S, Tsukamoto Y and Konoshima M (1988) A synthetic commentary on Iwasaki's "Honzozufu" II. Dohosha shuppan Inc, Kyoto : 1-1227.
Koyama T. (1987) Grasses of Japan and its neighboring regions. Kodansha Ltd, Tokyo : 1-570.
Kudo T, Uno H and Muramatsu M (1990) Intergeneric cross-compatibility and cytogenetics of hybrids in tribe Triticeae, Gramineae. I. Cross-compatibility and phenotype of the hybrids. Japan J Breed 40 (Suppl 2): 320-321 (in Japanese).
Kucera CL (1961) The Grasses of Missouri. University of Missouri Press Columbia (Missouri) : 1-241.
Maekawa F (1943) Prehistoric-naturalized plants to Japan proper. Act Phytotax Geobot XIII : 74-279. (in Japanese).
Makino T and Nemoto K (1931) Nippon-Shokubutsu-Soran (Flora of Japan) Second edition revised and enlarged. Shunyodo shoten, Tokyo : 1-1936 (in Japanese).
Matsumura J (1895). Shokubutsu Mei-I. Enumeration of selected scientific names of both native and foreign plants, with romanized Japanese names, and in many cases Chinese characters. Z.P. Maruya & Co (Maruzen kabushiki kwaisha), Tokyo : 1-321+62. (in Japanese).
Matsumura J (1905) Index Plantarum Japonicarum II.1: 1-299. (in Japanese). (Gramineae species excluding Bambuseae are described in pages from 33 to 87).
Matsumura S (1941) Interspecific hybrids in *Agropyron*. Kagaku 11: 245-248 (in Japanese).
Matsumura S (1942) Interspecific hybrid in *Agropyron*. II. Japan J Genet 18: 133-135 (in Japanese).
Matsumura S (1948) Genomanalyse bei *Agropyrum*, als verwandtschaftlicher Gattung von *Triticum* I. Artbastarde bei *Agropyrum*. Oguma Commem Vol, Cytol and Genet 1: 116-124. Hopposhuppan Co, Sapporo (in Japanese with German summary).
Matsumura S (1949) Genomanalyse bei *Agropyrum*, als verwandter Gattung von *Triticum*. II.Gattungsbastarde zwischen *Triticum* und *Agropyrum*. Japan J Genet Sup Vol 2: 35-44 (in Japanese with German summary).
Matsumura S and Muramatsu M (1956) F1 hybrid between three species of *Triticum* and *Agropyron intermedium*. Wheat Inf Serv 4: 13-14.
Matsumura S, Muramatsu M and Sakamoto S (1958a) Genome analysis in *Agropyron*, a genus related to *Triticum*. Proc Xth Int Cong Genet, Montreal: 181-182.
Matsumura S, Muramatsu M and Sakamoto S (1958b). Genome analysis in *Agropyron*, a genus related to *Triticum*. Seiken Ziho 9: 8-16.
Matsumura S and Sakamoto S (1955) Karyotypes of diploid *Agropyron* species. Wheat Inf Serv 2: 19.
Matsumura S, Sakamoto S and Tateoka T (1956) *Agropyron* and its related genera. In : Kihara H (ed) Land and crops of Nepal Himalaya. Scientific Results of the Japanese Expeditions to Nepal Himalaya, 1952-1953, II: 249-253. Fauna and Flora Research Soc, Kyoto Univ, Kyoto.
Muramatsu M (1955a) Karyological studies on the F1 hybrid between *Triticum aegilopoides* (autotetraploid) and *Agropyron intermedium*. Seiken Ziho 7: 75-85. (in Japanese with English summary).
Muramatsu M (1955b) A hybrid between autotetraploid *Triticum aegilopoides* and *Agropyron intermedium*. Wheat Inf Serv 2: 19-20.
Muramatsu M (1956) An F1 hybrid between *T. durum* 8x and *Agropyron trichophorum*. Wheat Inf Serv 3: 31.
Muramatsu M (1982) Chromosome pairing at meiotic prophase and MI of the F1 hybrid (*Agropyron tsukushiense* x *Triticum aestivum*). Japan J Genet 57: 689-690. (in Japanese).
Muramatsu M (1984) Chromosome pairing of the F1 hybrid between *Aegilops squarrosa* 4x and *Agropyron elongatum* (10x). Japan J Genet 59: 689-642. (in Japanese).
Muramatsu M (1985) Summer survival of the F1 plants that *Agropyron* species were crossed to wheat or rye, and result of the colchicine treatment of the F1. Japan J Breed 35 (Suppl 1): 350-351 (in Japanese).
Muramatsu M (1990) Cytogenetics of decaploid *Agropyron elongatum* (*Elytrigia elongata*) (2n=10x=70) I. Frequency of decavalent formation. Genome 31: 811-817.
Muramatsu M (1991) Cross-compatibility between distantly related genera in the family Gramineae, and species genetics. Shuseibutsu-gaku-kenkyu (Study of Species Biology) 15: 27-45 (in Japanese).
Muramatsu M (1992) Genetic studies on the Triticeae, Gramineae. III. F1 hybrids of crossing *Elymus* species indigenous to Japan as female to *Thinopyrum intermedium*. Japan J Breed 42 (Suppl 2): 422-423 (in Japanese).
Muramatsu M (1993) A survey on the species of the tribe Triticeae, Gramineae, distributed in Japan. Japan J Breed 43 (Suppl 2): 174 (in Japanese).
Muramatsu M (1994) Triticeae and Bambuseae species distributed in Japan and their characteristics as genetic resources. Sci Rep Fac Agr, Okayama Univ 83: 65-90 (in Japanese with English summary).
Muramatsu M (2001) Features of the F1 hybrids of *Hystrix longe-aristata* and two *Elymus* species. Hereditas 135: 115-118.
Muramatsu M and Ikeda R (1983) Variation of fertility and segregation of reaction to the powdery mildew inoculation in F1 and BF1 generations of the cross

involving hexaploid wheats and *Agropyron elongatum* (2n=10x=70). Japan J Genet 58: 665 (in Japanese).
Muramatsu M, Ikeda R, Uetsuki T and Kaneta S (1983) Intergeneric hybrids between the *Agropyron* species indigenous to Japan and hexaploid wheat, and their progeny generation 2. Hybridization study between the *Agropyron* species and hexaploid wheats and reaction of the F1 to wheat or *Agropyron* forma specialis of powdery mildew. Japan J Breed 33 (Suppl 1): 162-163 (in Japanese).
Muramatsu M and Kaneta S (1982) Intergeneric hybrids between the *Agropyron* species indigenous to Japan and hexaploid wheat, and their progeny generation. 1. Result of the cross and F1 hybrid production by embryo culture. Japan J Breed 32 (Suppl 2): 224-225 (in Japanese).
Muramatsu M, Kaneta S, Ikeda R, Uetsuki T and Takahashi K (1983) Hybridization of Japanese indigenous *Agropyron* (*Roegneria*) species with hexaploid wheat and cytogenetics of some of the F1, BF1 and amphiploid plants. Proc 6th Int Wheat Genet Symp, Kyoto Japan: 1041-1048.
Muramatsu M, Kumamoto Y, Takata K, Tamura N, Nakajima T, Horikawa M and Matsubara H (1988) Cross-compatibility of the Japanese indigenous *Agropyron* species with wheat or barley, and some of the F1 hybrids. Japan J Breed 38 (Suppl 1): 306-307 (in Japanese).
Muramatsu M, Nakatsuji R, Nagata M, Yanagihara K and Tonai N (1993) Cross-compatibility of *Elymus humidus* and F1 hybrid plants with *Triticum* and *Hordeum*. Sci Rep Fac Agr, Okayama Univ 81: 19-25.
Muramatsu M and Ohasa Y (1992) Some disomic addition lines of *Agropyron ciliare* chromosomes to *Triticum aestivum*. Sci Rep Fac Agr, Okayama Univ 80: 69-77 (in Japanese with English summary).
Muramatsu M and Sakamoto S (1956) F1 hybrids between three species of *Aegilops* and *Agropyron intermedium*. Wheat Inf Serv 4: 14-15.
Muramatsu M and Sigunga DO (1983) Amphiploid seed obtained by colchicine treatment of an F1 hybrid between *Triticum spelta* and *Agropyron intermedium*. Sci Rep Fac Agr, Okayama Univ 61: 1-3.
Muramatsu M and Takata K (1993) Frequency of disomic addition lines of *Agropyron ciliare* chromosome obtained in backcross generations of an amphiploid, *A. ciliare* x wheat. Sci Rep Fac Agr, Okayama Univ 81: 9-18 (in Japanese with English summary).
Muramatsu M, Takata K, Uno H, Kudo K, Ohe K, Hijiya K and Tsutsumi Y (1990) Intergeneric cross-compatibility of *Agropyron ciliare* (tribe Triticeae, Gramineae) and characters of the F1 hybrids, particularly appearance of cells with aberrant chromosome numbers. Japan J Breed 40 (Suppl 1): 332-333 (in Japanese).
Muramatsu M, Tamura N, Uno H, Kuioi N, Kudo T, Shioda T and Yamaguchi M (1989) Cross compatibility of two Japanese indigenous *Agropyron* species and cytogenetics of the F1 *A. tsukushiense* x *Hordeum vulgare*. Japan J Breed 39 (Suppl 1): 296-297 (in Japanese).
Muramatsu M, Tamura N, Uno H, Kuioi N, Kudo T, Shiota S, Yamaguchi M, Ohe K, Hijiya H, Tsutsumi H, Sorai M, Kashino N, Ohta T and Vu TT (1992) Cross-compatibility of Triticeae species indigenous to Japan and cytogenetics of F1 hybrids. Proc 1st Int Triticeae Symp, Helsingborg, Sweden, 1991. Hereditas (Offprint Vol) 116: 263-269.
Muramatsu M, Tonai N, Nagata M, Nakatsuji R and Yanagihara K (1992) Cross compatibility of *Elymus humidus* Osada. Japan J Breed 42 (Suppl 1): 412-413 (in Japanese).
Muramatsu M, Uno H, Kudo T, Sorai M, Kashino N, Ohta T and Vu TT (1991) Intergeneric cross-compatibility in the tribe Triticeae, Gramineae, particularly on the chromosome instability of the F1 hybrids. Japan J Breed 41 (Suppl 1): 120-121 (in Japanese).
Nagano Prefecture Flora Compilation Committee (1997) Shimizu T (ed) Flora of Nagano Prefecture. The Shinano Mainichi Shinbun (Newspaper) Inc, Nagano (Japan) : 1-1735.
Nakajima G (1936) Chromosome numbers in some crops and wild angiosperms. Japan J Genet 12: 211-218.
Nakatsuji R, Nagata M and Muramatsu M (1992) Genetic studies on the Triticeae, Gramineae. I. Chromosome instability of the F1 hybrid between *Elymus humidus* and barley, and occurrence of polyhaploid plants. Japan J Breed 42 (Suppl 2): 420-421 (in Japanese).
Nishiyama I (1954) Cytogenetics of wheat and its closely related genera. In: Kihara (ed) Studies of wheat, Revised edit : 435-586 Yokendo Ltd, Tokyo (in Japanese).
Ohwi J (1941) Gramina Japonica 1. Act Phytotax et Geobot 10: 94-135 (in Japanese).
Ohwi J (1942) Gramina Japonica IV. Act Phytotax et Geobot 11: 145-193 (in Japanese).
Ohwi J (1953) Frora of Japan. Shibundo, Tokyo : 1-1383 (in Japanese).
Ohwi J (1965) Flora of Japan. Rivised edition, Shibundo Tokyo: 1-1560 (in Japanese).
Ohwi J (1983) New Flora of Japan (revised by Kitagawa M) Shibundo, Tokyo : 1-1716.
Ohwi J (1984) Flora of Japan (in English). Meyer FG and Walker EH (English ed), Smithsonian Institution, Washington DC : 1-1067.
Ohwi J and Sakamoto S (1964) Taxonomy, ecology and distribution of *Agropyron humidum* Ohwi et Sakamoto. J Japan Bot 39 (4): 109-114.
Okamoto M (1957) Asynaptic effect of chromosome V. Wheat Inf Serv 5 : 6.
Osada T (1972) Illustrated Japanese alien plants. Hokuryukan Co Ltd, Tokyo : 1-254.
Osada T (1989) Illustrated grasses of Japan. Heibonsha Ltd, Tokyo: 1-759 (in Japanese and English).
Osada T (1993) Illustrated grasses of Japan. Enlarged edition. Heibonsha Ltd, Tokyo : 1-777 (Japanese and English)
Sakamoto S (1959) A new early ecotype of *Agropyron tsukushiense* var. *transiens* Ohwi. Wheat Inf Serv 8: 8-10.
Sakamoto S (1961) An early ecotype of *Agropyron tsukushiense* var. *transiens*. Seiken Ziho 12: 45-48 (in Japanese with English summary).
Sakamoto S (1964a) Cytogenetic studies in the tribe Triticeae. I. A polyhaploid plant of *Agropyron tsukushiense* var. *transiens* Ohwi found in a state of nature. Japan J Genet 39: 393-400.
Sakamoto S (1964b) Cytogenetic problems in *Agropyron* hybrids. Pro 4th Wheat Symp, Japan. Seiken Ziho 16: 38-47.
Sakamoto S (1965) Morphological and cytological studies of two intergeneric hybrids of *Elymus sibiricus* Linn. and *El. dahuricus* Turcz. with *Agropyron tsukushiense* var. *transiens* Ohwi. Nat Inst Genet Ann Rep 15: 79.
Sakamoto S (1966) Cytogenetic studies in the tribe Triticeae. IV. Natural hybridization among Japanese *Agropyron* species. Japan J Genet 41 (3): 189-201.
Sakamoto S (1967a) Genome analysis of the genus *Eremopyrum*. Wheat Inf Serv 23-24: 21-22.
Sakamoto S (1967b) Cytogenetic studies in the tribe Triticeae. V. Intergeneric hybrids between two *Eremopyrum* species and *Agropyron tsukushiense*.

Seiken Ziho 19: 19-27.
Sakamoto S (1968) Cytogenetic studies in the tribe Triticeae. VI. Intergeneric hybrids between *Eremopyrum orientale* and *Aegilops squarrosa*. Japan J Genet 43 :167-171.
Sakamoto S (1969) Interspecific hybrid between the two species of the genus *Taeniatherum* of the tribe Triticeae. Wheat Inf Serv 28: 26-27.
Sakamoto S (1971) Intergeneric hybrid between *Agropyron tsukushiense* and *Elymus mollis*. Nat Inst Genet Ann Rep 21: 97.
Sakamoto S (1972) Intergeneric hybridization between *Eremopyrum orientale* and *Henrardia persica*, an example of polyploid species formation. Heredity 28: 109-115.
Sakamoto S (1973) Patterns of phylogenetic differentiation in the tribe Triticeae. Seiken Ziho 24: 11-31.
Sakamoto S (1974) Intergeneric hybridization among three species of *Heteranthelium*, *Eremopyrum* and *Hordeum*, and its significance for the genetic relationships within the tribe Triticeae. New Phytol 73: 341-350.
Sakamoto S (1978) Adaptation of two Japanese *Agropyron* species to the winter fallow paddy fields. Weed Res (Japan) 23 (3) : 101-108 (in Japanese).
Sakamoto S (1982) Cytogenetical studies on artificial hybrids among *Elymus sibiricus*, *E. dahuricus* and *Agropyron tsukushiense* in the tribe Triticeae, Gramineae. Bot Mag Tokyo 95: 375-383.
Sakamoto S (1984) Mizuta-kamojigusa-ni-tsuite (On the species, *Agropyron humidum* Ohwi et Sakamoto). Hukuoka-no-shokubutsu (Plants of Hukuoka) 10: 1-8 (in Japanese).
Sakamoto S and Muramatsu M (1962) Chromosome number of Gramineae species collected in Pakistan, Afghanistan and Iran. Chromosome Inf Serv 3: 32-33.
Sakamoto S and Muramatsu M (1963) Preliminary studies on the relationship of diploid *Eremopyrum distans* and *E. triticeum* (Gramineae). Can J Genet Cytol 5: 433-436.
Sakamoto S and Muramatsu M (1965) Morphological and cytological studies on various species of Gramineae collected in Pakistan, Afghanistan and Iran. In: Yamashita K (ed) Cultivated Plants and Their Relatives. Results of the Kyoto Univ Sci Exp to the Karakoram and Hindukush (1955) I: 119-140.
Sakamoto S and Muramatsu M (1966a) Cytogenetic studies in the tribe Triticeae. II. Tetraploid and hexaploid hybrids of *Agropyron*. Japan J Genet 41: 155-168.
Sakamoto S and Muramatsu M (1966b) Cytogenetic studies in the tribe Triticeae. III. Pentaploid *Agropyron* hybrids and genomic relationships among Japanese and Nepalese species. Japan J Genet 41: 175-187.
Satake Y, Ohwi J, Kitamura S, Watari S and Tominari T (1982) (ed) Wild floweres of Japan. Herbaceous plants (including dwarf subshrubs) Heibonsha, Tokyo : 1-305 (in Japanese).
Sears ER (1941) Amphidiploids in the seven-chromosome Triticinae. Res Bull Mo Agric Exp Sta 336: 1-46.
Shigenobu T and Sakamoto S (1981) Intergeneric hybridization between *Agropyron tsukushiense* and *Hordeum bulbosum* (4x). Japan J Genet 56: 505-517.
Shigenobu-Kishimoto T and Sakamoto S (1982) Haploid and intergeneric hybrid production in the tribe Triticeae pollinated by *Hordeum bulbosum*. II. Japan J Breed 32 (Suppl 1): 144-145 (in Japanese).
Shigenobu-Kishimoto T and Sakamoto S (1984) An aneuploid (2n=15) *Agropyron elongatum* (2x) obtained from intergeneric crosses with *Hordeum bulbosum* (4x). Japan J Breed 34 (Suppl 2): 314-315 (in Japanese).
Shiotani I (1968) Species differentiation in *Agropyron*, *Elymus*, *Hystrix* and *Sitanion*. Proc XII. Intern Congr Genet 1: 184.
Steyermark JA (1981) Flora of Missouri. The Iowa State University Press, Ames Iowa : 1-1728.
Tkahashi, H, Katsuyama T and Tanaka N (2003) (ed) Plants of Yokohama in nature and human life. The Botany Club of Yokohama, Yokohama : 1-1325 (in Japanese).
Takahashi K and Muramatsu M (1981) Intergeneric F1 hybrid between *Agropyron tsukushiense* and *Triticum aestivum*. Japan J Breed 31 (Suppl 1): 116-117 (in Japanese).
Takata K and Muramatsu M (1992) Genetic studies on the Triticeae, Gramineae. II. Production of disomic addition lines of *Elymus cilliaris* chromosomes. Japan J Breed 42 (Suppl 2): 368-369 (in Japanese).
Turesson G (1922) The genotypical response of the plant species to the habitat. Hereditas 3: 211-350.
Uno H, Kudo T and Muramatsu M (1990) Intergeneric cross-compatibility and cytogenetics of hybrids in the tribe Triticeae, Gramineae. II. Somatic chromosome number aberration and variation. Japan J Breed 40 (Suppl 2): 322-323 (in Japanese).
Uno H and Muramatsu M (1989) Results of the colchicine treatment of the F1 plants between *Agropyron ciliare* and wheats. Japan J Breed 39 (Suppl 2): 414-415 (in Japanese).
Walker EH (1976) Flora of Okinawa and the Southern Ryukyu Islands. Smithsonian Institution Washington DC: 1-1159 (in Japanese with English summary).
Watanabe Y and Mukade K (1957) Cytogenetical studies on the intergeneric hybrids between *Triticum* and *Agropyron*. I. The meiotic behaviour and the morphology of F1 hybrids between *Triticuim vulgare* Vill and *Agropyron glaucum* Roem et Schult. Japan J Genet 32: 136-145.
Winkle ME and Kimber G (1976) Colchicine treatment of hybrid in the Triticinae. Cereal Res Commun 4: 317-320.
Wolfe JA (1979) Temperature parameters of humid to mesic forests of eastern Asia and relation to forests of other regions of the northern hemisphere and Australasia. Geological survey professional paper 1106. United States Government Printing Office, Washington DC : 1-37.
Yokouchi I (1983) Shinano shokubutsushi (Flora of Shinano). Gingashobo, Nagano (Japan) : 1-400 (in Japanese).
Yuhki Y (1992) New Flora of Yamagata. Yamagataken-no-shokubutsushi-kankouiinkai (Committee for publication of New Flora of Yamagata) : 1-487 (in Japanese).

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A *scs*^{ae} gene on chromosome 1D alters the expression of a gametocidal (*scs*^{spt}) gene in a T2BL.2S chromosome

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Summary

A species cytoplasm specific gene (*scs*^{ae}) on the long-arm of chromosome 1D (1DL) from common wheat (*Triticum aestivum* L.) produced compatibility of the nuclear genome of durum wheat (*T. turgidum* L.) with the cytoplasm of D- or M-genome *Aegilops* species, including an altered cytoplasm of *Ae. longissima* (*lo*). Similarly, a 29-chromosome durum line with *Ae. uniaristata* (*un*) cytoplasm and a *un*-telocentric homoeologous to group 4 chromosomes, a (*lo*) durum line with *scs*^{ti} in the long-arm of chromosome 1A from *T. timopheevi*, and a (*Ae. ventricosa*) durum [(*vent*) line] with *scs*^{spt} in T2BL.2S translocation chromosome having 2S from *Ae. speltoides* were compatible but male-sterile. Our objective was to determine the transmission of female gametes with *scs*^{ae} versus those with *scs*^{ti}, *scs*^{un}, or *scs*^{spt}. We crossed (*lo*) *scs*^{ti}, (*un*) *scs*^{un}, and (*vent*) *scs*^{spt} durum lines to 1D(1A) and 1D(1B) disomic-substitution lines of Langdon durum (LDN DS) and crossed hybrid progeny to the corresponding paternal LDN DS lines as recurrent male parents. The resulting alloplasmic LDN DS lines were male sterile and when crossed to paternal LDN DS lines; (*lo*) and (*un*) DS lines had complete seed-set and 14 closed chromosome pairs, while (*vent*) DS lines had partial seed-set, 13 closed chromosome pairs and 1 open pair at the meiotic metaphase I in the pollen mother cells. The results showed that *scs*^{ae} replaced *scs*^{ti} or *scs*^{un} in the (*lo*) and (*un*) DS lines, that 2S did not pair with 2BS, and that *scs*^{spt} was expressed as a gametocidal (Gc) gene but did not produce chromosomal structural abnormalities in the (*vent*) 1D(1A) and 1D(1B) DS lines having *scs*^{ae} in chromosome 1D.

Key words: interactions among the *scs*^{ae} and *scs*^{ti}, *scs*^{un}, or *scs*^{spt} genes, interspecific nucleo-cytoplasmic compatibility.

Introduction

According to the species cytoplasm specific (*scs*) gene hypothesis (Maan 1995), the native *scs* genes produce intraspecific nucleo-cytoplasmic compatibility and male fertility but are expressed as male sterility when the nuclear genome of one species is substituted in the cytoplasm of another by the backcross method of Kihara (1951). The resulting male-sterile lines are maintained by crossing to normal counterparts. Similarly, the parental *scs* genes are essential components of interspecific sterility when in the hemizygous condition in the species hybrids but add to the fertility when in the homozygous condition in the amphidiploid progeny.

The nuclear genome of common wheat (*Triticum aestivum* L.; 2n=6x=42, 21", AABBDD) is compatible, but the nuclear genome of durum wheat (*T. turgidum* L.; 2n=4x=28, AABB) is partially or completely incompatible with the cytoplasm of D- and M-genome *Aegilops* species (Maan 1975; 1978), including an experimentally altered cytoplasm of *Ae. longissima* (2n=14, 7", SS) (Maan 1996). A species cytoplasm

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specific gene (*scs*^{ae}) on the long-arm of chromosome 1D (1DL) from common wheat improved compatibility between durum wheat and the alien cytoplasms, *Ae. longissima*, *Ae. uniaristata* (*un*) (2n=14, 7", M^{u}M^{u}), and *Ae. ventricosa* (*vent*) (2n=28, 14", DDMM) (Maan and Endo 1981, 1991; Tsuji and Maan 1981). The resulting 29-chromosome durum lines with 1D were male sterile and when crossed to normal durum produced plump and viable seeds having 29-chromosome embryos with 1DL, while seeds having 28-chromosome embryos (without 1DL) were shrivelled and inviable or they produced male-sterile plants of greatly reduced vigor (Maan 1975, 1978, 1992a, b, c, 1994).

Nucleo-cytoplasmic interactions involving alien cytoplasms and nuclear genomes of durum wheat and common wheat alter the expression of wheat nuclear genes and produce a variety of phenotypes, including reduced male and/or female fertility, delayed maturity, reduced plant vigor, and floral abnormalities in the alloplasmic wheat lines (Kihara 1951; Maan 1975, 1978). The species cytoplasm specific (*scs*) genes producing nucleo-cytoplasmic compatibility may mutate cytoplasm (Maan 1996), or modify the expression of other *scs* genes in the euplasmic (Kihara 1951, 1982) or alloplasmic wheat lines. For example, R5, an elite R-line of common wheat produced fertile F_1 and F_2-derived advanced generation progeny that included fertile lines with greatly reduced plant vigor and fertile lines with normal vigor plants (Gomaa 1973; Gomaa and Lucken 1973; Maan unpubl), indicating that the R5 had mutant *scsscs* and *ViVi* (vitality) in addition to the male fertility restorer (*Rf*) genes. Similarly, a gene for black color glumes in a *Ae. caudata* chromosome homeologous group 1 produced fertility in (*Ae. caudata*) common wheat line and frequently mutated and produced non-black glumes when hemizygous in the cytoplasmic background of normal common wheat (Kihara 1951, 1982).

Similarly, *Vi*, a gene mutant at the latent Rf_2 locus in the short arm of chromosome 1B of durum wheat, chromosome 1B of Chinese Spring wheat (Maan unpubl) and *T. spelta* var. *duhamelianum* (Tahir and Tsunewaki 1969) had a positive zenia effect on seed plumpness and produced a male fertile (*lo*) 29-chromosome durum line having *scs*^{ae} in 1DL and a (*un*) 29-chromosome durum line having *scs*^{un} on *un*-telocentric homoeologous to group 4 chromosomes (Maan unpubl), while seeds having 28-chromosome embryos (without *scs*^{ae} or *scs*^{un}) produced partially fertile plants of greatly reduced vigor (Maan 1992a, b, c, 1994). Also, *Vi* produced fertility in a (*lo*) durum line (2n=28, 14") having a *scs*^{ti} gene from *T. timopheevi* Zhuk. (2n=28, 14", AAGG) located in the long arm of chromosome 1A, while seeds with *Vi* but without *scs*^{ti} produced partially fertile plants of greatly reduced vigor (Anderson and Maan 1995).

Tsujimoto and Tsunewaki (1988) reported that common wheat having short arm of chromosome 2S from *Ae. speltoides* (homoeologous to 2BS of common wheat) had a gametocidal (*Gc*) gene. Similarly, Maan and Kianian (2001) reported that a *scs*^{spt} gene on a T2BL.2S translocation chromosome consisting of the short arm of chromosome 2S from *Ae. speltoides* and the long arm of chromosome 2B of durum wheat produced compatibility with (*vent*) cytoplasm and the resulting (*vent*) *scs*^{spt} durum line was male sterile, *scs*^{spt} was expressed as a gametocidal (*Gc*) gene, and produced chromosomal structural abnormalities in the (*vent*) durum line.

Our objective was to determine the relative transmission of *scs*^{ae} versus *scs*^{ti}, *scs*^{un}, and *scs*^{spt} genes through the female gametes of (*lo*), (*un*) and (*vent*) durum lines, respectively.

Materials and methods

A (*vent*) durum line having a T2BL.2S translocation chromosome consisting of long arm of chromosome 2B (2BL) of durum and the short arm of chromosome 2S (2S) of *Ae. speltoides*, a (*lo*) 28-chromosome durum line having *scs*^{ti} from *T. timopheevi*, and (*un*) 29-chromosomes having a *un* telo were available at North Dakota State University, Fargo, ND. These lines were used as female parents. The 1D(1A) and 1D(1B) disomic-substitution (DS) lines of Langdon durum were available from Joppa (1988).

The (*vent*) 28-chromosome T2BL.2S/2B durum, (*lo*) 28-chromosome *scs*^{ti} durum, and (*un*) 29-chromosome (2n=29, 14"+t' (*un*-telo)) durum lines were crossed to the 1D(1A) and 1D(1B) DS lines of Langdon durum (LDN). The resulting 1D+1A and 1D+1B double-monosomic (dM) F_1s were backcrossed to the corresponding LDN DS lines as the recurrent male parents. The resulting 1D(1A) and 1D(1B) DS lines of LDN durum were maintained by crossing to the corresponding LDN DS lines. The crossed spikes of the alloplasmic 1D(1A) and 1D(1B) DS lines were examined for seed-set. The individual plants of the hybrid progeny were examined for meiotic chromosome number and meiotic chromosome pairing at metaphase I of meiosis in the pollen mother cells (PMC).

Table 1. Meiotic chromosome pairing and seed-set in alloplasmic 1D(1A) and 1D(1B) disomic-substitution (DS) lines from crosses to the corresponding 1D(1A) or 1D(1B) DS lines of Langdon durum

Alloplasmic DS x 1D(1A) or 1D(1B) DS lines†	Mean seed-set in primary spikes of 10 plants	Meiotic chromosome pairing	Genotype‡
(*vent*) 1D(1A)	17.1	13"+1" open pair	(*vent*) $scs^{spt}-$, scs^{ae} scs^{ae}
(*vent*) 1D(1B)	18.1	13"+1" open pair	(*vent*) $scs^{spt}-$, scs^{ae} scs^{ae}
(*lo*) *scs* 1D(1A)	33.5	14" closed pair	(*lo*) scs^{ae} scs^{ae}
(*lo*) *scs* 1D(1B)	34.5	14" closed pair	(*lo*) scs^{ae} scs^{ae}
(*un*)1D(1A)	39.0	14" closed pair	(*lo*) scs^{ae} scs^{ae}
(*un*)1D(1B)	35.0	14" closed pair	(*lo*) scs^{ae} scs^{ae}
LDN 1D(1A)	42.0	14" closed pair	(*lo*) scs^{ae} scs^{ae}
LDN 1D(1B)	50.0	14" closed pair	(*lo*) scs^{ae} scs^{ae}

† (*vent*), (*lo*), and (*un*) are cytoplasm from *Ae. ventriocsa*, *Ae. longissima*, and *Ae. uniaristata*, respectively.
‡ (*vent*) 1D(1A) and (*vent*) 1D(1B) DS lines are hemizygous for scs^{spt} and homozygous for scs^{ae}, while (*lo*) and (*un*) DS lines are homozygous for scs^{ae} scs^{ae} gene pair.

Results and Discussion

The backcrosses of (*lo*) or (*un*) durum lines to the LDN 1D(1A) and 1D(1B) DS lines as recurrent male parents produced (*lo*) or (*un*) 1D(1A) and 1D(1B) DS lines that were male sterile. They had complete seed-set in the crossed spikes, 14 closed pairs (13 pairs of durum chromosomes and 1 pair of 1D chromosomes) in the PMCs and were maintained by crossing to the corresponding LDN DS lines. Apparently, the scs^{ae} on chromosome 1D substituted for scs^{ti} in the (*lo*) scs^{ti} durum, and for scs^{un} in the (*un*) scs^{un} durum lines (Table 1).

The backcrosses of (*vent*) scs^{spt} durum having T2BL.2S translocation chromosome to the LDN 1D(1A) and 1D(1B) DS lines as recurrent male parents also produced male sterile (*vent*) 1D(1A) and 1D(1B) DS lines that were maintained by crossing to the corresponding LDN 1D(1A) or 1D(1B) DS lines (Table 1). However, compared to (*lo*) or (*un*) DS lines, the (*vent*) DS lines produced only about 50% of the seed set in the crossed spikes.

The meiotic chromosome pairing in the PMCs of plants in the (*vent*) 1D(1A) and (*vent*) 1D(1B) DS lines showed that they had 13 closed chromosome pairs and 1 open pair, indicating that the female gametes with T2BL.2S were exclusively transmitted and gametes without this chromosome did not function. However, scs^{spt} did not produce translocations in the presence of scs^{ae} on chromosome 1D, even though the scs^{spt} produced chromosomal structural alterations indicated by one or more multivalent configurations in the PMCs of certain plants of the (*vent*) scs^{spt} durum line (Maan and Kianian 2001). The (*vent*) 1D(1A) and (*vent*) 1D(1B) DS lines having scs^{spt} were partially female fertile and produced 50% seed set in the crossed spikes relative to *un* or *lo* crosses.

The results indicated that (*vent*) durum having T2BL.2S translocation chromosome with 2S from *Ae. speltoides* produced functional female gametes having T2BL.2S and those without this chromosome did not function. The T2BL.2S chromosome remained fixed in the (*vent*) 1D(1A) and 1D(1B) DS lines but did not produce chromosomal abnormalities. Possibly, the expression of scs^{spt} was altered in the presence of $scs^{ae}scs^{ae}$ gene in chromosome 1D pair of the (*vent*) 1D(1A) and (*vent*) 1D(1B) DS lines of LDN durum.

References

Anderson JA and Maan SS (1995) Interspecific nuclear-cytoplasmic compatibility controlled by genes on group 1 chromosomes in durum wheat. Genome 38: 803-808.

Gomaa ASA (1973) Genetics of male-fertility and plant vigor restoration in common wheat with cytoplasms from *Triticum timopheevi* and *T. boeoticum*. PhD Thesis, N Dak State Univ, Fargo, ND, USA.

Gomaa ASA and Lucken KA (1973) Male-fertility and plant-vigor restoration in common wheat with *Triticum timopheevi* and *T. boeoticum* cytoplasm. Proc 4th Int Wheat Genet Symp: 345-350.

Joppa LR (1988) Cytogenetics of tetraploid wheat. Proc 7th Int Wheat Genet Symp, Cambridge: : 197-202.

Kihara H (1951) Substitution of nucleus and its effects on genome manifestations. Cytologia 16: 177-193.

Kihara H (1963) Nucleus and chromosome substitution in wheat and *Aegilops*, II. Chromosome Substitution. Seiken Zihô 15: 13-23

Kihara H (1982) Wheat studies. Kodansha LTD. Elsevier Scientific Publishing Co., Amsterdam-Oxford-New York: pp 2308.

Maan SS (1975) Cytoplasmic variability and speciation in *Triticinae*. In Wali KM (ed) Prairie; A multiple view. Univ. N. Dak. Grand Forks, ND: 255-281.

Maan SS (1978) Cytoplasmic relationships among the D- and M-genome *Aegilops* species. Proc 5th Int. Wheat Genet Symp, New Delhi: 232-250.

Maan SS (1992a) Transfer of a species-cytoplasm-specific (*scs*) gene from *Triticum timopheevi* to *T. turgidum*. Genome 35: 238-243.

Maan SS (1992b) A gene for embryo-endosperm compatibility and seed viability in alloplasmic *Triticum turgidum*. Genome 35: 772-779.

Maan SS (1992c) The *scs* and *Vi* genes correct a syndrome of cytoplasmic effects in alloplasmic durum wheat. Genome 35: 780-787.

Maan SS (1994) Interactions between the *scs* and *Vi* genes in alloplasmic durum wheat. Genome 37: 210-216.

Maan SS (1995) The species-cytoplasm-specific hypothesis. In Gill BS (ed) Proc. Classical and Molecular Cytogenetic Analysis of Cereals Genomes. US-Japan Joint Seminar, Kansas State Univ, Manhattan, Kansas, USA :163-184.

Maan SS (1996) Natural or induced nucleocytoplasmic heterogeneity in *Triticum longissimum*. Genome 39: 71-76.

Maan SS and Endo TR (1981) Cytoplasmic homology between diploid *Aegilops uniaristata* and related polyploids, *Ae. crassa*, *Ae. juvenalis*. Cereal Res. Comm. 9: 9-15.

Maan SS and Endo TR (1991) Nucleo-cytoplasmic interactions stabilize ploidy level in wheat interspecific hybrids. Genome 34: 983-987.

Maan SS and Kianian SF (2001) An *Aegilops speltoides*-derived scs^{spt} gene located in T2BL.2S translocation chromosome of durum wheat. Wheat Inf Serv 93: 14-18.

Tahir ChM and Tsunewaki K (1969) Monosomic analysis of *T. spelta* var. *duhamelianum*, a fertility restorer for *T. timopheevi* cytoplasm. Jap J Genet 44: 1-9.

Tsuji S and Maan SS (1981) Differential fertility and transmission of male and female gametes in alloplasmic wheat. Can J Genet Cytol 23: 337-348.

Tsujimoto H and Tsunewaki K (1988) Gametocidal genes in wheat and its relatives. III. Chromosomal location and its effects of two *Aegilops speltoides*-derived gametocidal genes in common wheat. Genome 30: 239-244.

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Illustration of and a key to the genus *Triticum* L. in Iran

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Summary

The genus *Triticum* in Iran is illustrated and revised taxonomically. A total of 24 herbarium specimens and 245 accessions collected from the field were examined morphologically. The taxonomic literature of *Triticum* in Iran is reviewed and discussed. In total, nine *Triticum* species including three diploid species (*T. urartu* Thum. ex Gandil., *Triticum monococcum* L. and *T. boeoticum* Boiss.), four tetraploid species (*T. dicoccum* Schrank. ex Schübl., *T. dicoccoides* (Körn ex Aschers. et Graebn.) Thell, *T. turgidum* L. and *T. durum* Desf.) and two hexaploid species (*T. aestivum* L. and *T. compactum* Host.) were identified. Root tip mitosis was observed and chromosome counts made on all accessions collected from the field. A biometrical evaluation was performed on 160 morphological characters. A taxonomic brief key to the genus *Triticum* in Iran is presented along with a line drawing of each species. The geographical distribution of the species in Iran is summarized on a map. Two new species, *T. urartu* (2n=14) and *T. compactum* (2n=42), are reported for the first time for the flora of Iran.

Key words: *Triticum* L., taxonomy, Iran

Introduction

The genus *Triticum* is vitally important economically and has always been the focus of taxonomic and biosystematic attention. Allopolyploidy along with natural and artificial hybridization, as well as nomenclatural debates, have blurred both biological and taxonomic concepts of the species in this and closely related genera (Gupta and Baum 1989; Morrison 1993; Gupta 1997; Yen et al. 1997; Morrison and Raupp 1999). Morrison (1993) pointed out, that "many current treatments of *Triticum*, including the floras for the regions of western and central Asia where the wheats are endemic, are lacking in consistency and informational content."

Although the wild germplasm of *Triticum* in Iran, which is one of the important centers of origin and diversity for different ploidy levels of cultivated wheats (Vavilov 1992), is of immense importance, the taxonomic status of the genus in this country has not been studied adequately (Table 1). Bor (1970), in his treatment in Flora Iranica, mentioned 14 species of which eleven were recorded in Iran itself.

Changing taxonomy of the tribe *Triticeae* due to increasing information and changing names caused by nomenclatural rules (Gupta and Baum 1989) have greatly affected the taxonomy of the genus *Triticum*. The deficiency of taxonomic treatments has resulted from inadequate collections; poorly constructed keys and lack of sufficient information in the descriptions, as easily observable in the local taxonomic treatments (Morrison 1993).

This study aimed to make as complete a germplasm collection as possible from Iran, and carry out a thorough taxonomic treatment of *Triticum* taxa occurring in this country.

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Materials and methods

The materials consisted of field collections (Fig. 1) made as seed accessions and herbarium specimens, or the herbarium specimens from different herbaria of Iran (Table 2). Taxonomic studies were performed on both herbarium specimens and the field materials. Each accession was provided with a voucher specimen deposited at the herbarium of Isfahan University.

In order to gain a general idea of the morphological characters in the genus, 160 morphological characters were studied. Of these, eight quantitative and qualitative characters were selected as the most informative ones for this study. The characters studied are listed in Table 3. Morphological descriptions are based on Bor (1970) and Stearn (1983). For flag leaf width and length, 30 individuals for each population were examined. Mean and cv. (coefficient of variation) were calculated for each species. In order to avoid misidentifications caused by the deficiency of taxonomic descriptions and poorly constructed keys, particularly in the case of *T. aestivum*, *T. turgidum* and *T. durum*, a cytotaxonomic survey was performed on all accessions examined in this study. For each accession 1–3 root tips were studied (Waines and Kimber 1973; Lange and Jochemzen 1992).

The names and authorities of the diploid species and of *T. dicoccoides* are taken from Van Slageren (1994), and those of the tetraploid and hexaploid

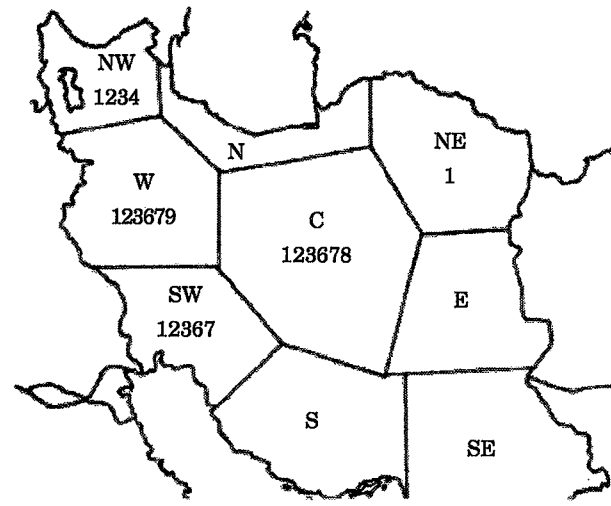

Fig. 1. Distribution of *Triticum* spp. in Iran. The zone determined on the map associated the climatic and bio-geographic conditions as, N: north, S: south, W: west, E: east, SE: south east, NW: north west, NE: north east, SW: south west. Numbers in the zones are associated to the species as; 1: *T. aestivum*, 2: *T. durum*, 3: *T. turgidum*, 4: *T. compactum*, 5: *T. dicoccum*, 6: *T. boeoticum*, 7: *T. monococcum*, 8: *T. urartu*, 9: *T. dicoccoides*.

Table 1. A comparison of *Triticum* spp. reported for Iran, between four main floras and the results of this study.

Species	Boissier 1885	Parsa 1954	Bor 1970	Mobayen 1980	Results of this study
T. aestivum L.	-	+	+	+	+
T. boeoticum.	+	-	+	+	+
T. compactum	-	-	-	-	+
T. dicoccoides	-	+	+	+	+
T. dicoccum	-	+	+	+	+
T. durum	-	+	+	+	+
T. hamosum Rehm. ex Boiss	+	-	-	-	-
T. monococcum	-	+	+	-	+
T. orientale	-	+	+	+	-
T. persicum Vav.	-	+	-	-	-
T. polonicum	-	+	+	+	-
T. spelta	-	-	+	+	-
T. thaoudar Reut.	+	+	-	-	-
T. turgidum	-	+	+	+	+
T. urartu	-	-	-	-	+

species from Bor (1970). Drawings are based on the herbarium sheets deposited in the herbarium of Isfahan University (Fig. 2); the code number of each sheet used for drawing is designated as Tr- followed by the number of wheat collection in the above herbarium.

Results and discussion

The results of this study show that the material belongs to three ploidy levels and nine species: three diploid species (2n=2x=14), *T. urartu* Thum. ex Gandil., *Triticum monococcum* L. and *T. boeoticum* with subsp. *boeoticum* Boiss. ex Hausskn. and subsp. *thaoudar* (Reut. ex Hausskn.) Schiem.; four tetraploid

Table 2. Number of accessions and locality features of the populations examined in this study.

Species	No. of accessions	Locality and altitude(m)	Herbarium
T. aestivum			
	10†	Bakhtiari (1600-2100)	
	11	Isfahan (2200-2450)	TARI 1‡
	11	Lorestan (2080-2250)	
	2	Khuzistan (450-880)	
	1	Ilam (1120-1520)	
	20	Kermanshah (1120-1850)	
	1	Tehran (1000)	TARI 3
	1	Markazi (2000)	
	6	Kurdestan (1500-1560)	
	37	West of Azerbaijan (1270-1650)	TARI 3
	6	East of Azerbaijan (1400-1670)	
	1	Hamadan (1500)	
T. durum			
	4	Kohkiloye & Boyerahmad (1550-2370)	THU 1
	11	Khuzistan (450-880)	
	10	Lorestan (850-1120)	
	5	Bakhtiari (1900-2000)	
	9	Kermanshah (1350-1720)	
	2	Kurdestan (1200-1660)	
	3	Kohkiloye & Boyerahmad (1550-2370)	TARI 2
	5	Khouzistan (450-880)	
T.compactum			
	1	West of Azerbaijan (1400-1500)	
T. dicoccum			
	1	West of Azerbaijan (1400)	
T. turgidum			
	1	Lorestan(1120)	TARI 1
	3	Kohkiloye & Boyerahmad (1550-2370)	TARI2
	15	Bakhtiari (1940-2130)	
	5	Khuzistan (450- 880)	
	4	Kermanshah (1330-1340)	
	4	Kurdestan (1620-1880)	
T. boeoticum			
	1	Isfahan (2210)	
	7	Kohkiloye & Boyerahmad (1650-2050)	
	6	Lorestan (1100-1820)	
	10	Bakhtiari (1980-2090)	
	7	Kermanshah (1330-1770)	TARI 1, IRAN 3
	4	West of Azerbaijan (950-1390)	
	2	Tehran (900)	IRAN 1
T. monococcum			
	1	West of Azerbaijan (1500)	TARI 1
	1	Kohkiloye & Boyerahmad (2100)	
	1	Markazi (2020)	
	2	Tehran (1850)	TARI 1
	1	Kermanshah (1340-1480)	TARI 1
	1	Lorestan (1250)	
	1	East of Azerbaijan (1410)	
	1	Kurdestan (1400)	
T. urartu			
	2	Kermanshah (1480)	
	3	West of Azerbaijan (1500)	
	1	Bakhtiari (2080)	TARI 1
T. dicoccoides			
	1	Kermanshah (1480)	
	1	Isfahan (1850)	

†The number underneath of each species shows the number of accessions investigated. For the examined accessions a herbarium sheet is deposited in the herbarium of Isfahan University.

‡ the abbreviations as: TARI: Research Institute of Forest and Ranglands, Tehran, THU: Tehran University, IRAN: Plant and Disease Research Institue of Evin (Holmgren et al. 1990).

Table 3. The characters that studied in genus of *Triticum* in Iran.

Species	Flag leaf (cm)				Indumentum of leaf	Color of node	Indumentum of glume	Form of spike	Endosperm texture	Color of glume
	Lengh		Width							
	Mean	cv.	Mean	cv.						
Taestivum	21.7	17.4	0.53	3.4	Hairy, without hair	Yellow & green, Yellow & brown	Hairy, without hair	Ovate	Mealy & sub mealy	Brown & yellow, Green & yellow Green & brown, Yellow & black
T.turgidum	24.6	28.6	0.6	1.8	Hairy, without hair	Yellow & green, Yellow & brown	Hairy, without hair	Ovate	Mealy & sub mealy	Green & brown, Green & yellow, Yellow
T.durum	24.1	15.1	0.6	2.4	Without hair	Yellow & green	Hairy, without hair	Quadrate	Flinty & sub flinty	Yellow & green, Yellow & brown, Brown
T.compactum	13.1	49.1	0.67	2.1	Hairy	Yellow & brown	Hairy	Quadrate	Mealy	Brown & yellow
T.dicoccoum	29.3	13.5	0.57	2.3	Without hair	Yellow & brown	Hairy, without hair	Cylindrical	Flinty & sub flinty	Yellow
T.boeoticum	6.8	3.5	0.4	1.2	Very hairy	Purple, brown	Hairy, without hair	Cylindrical	Sub mealy	Yellow & brown, Black & yellow
T.monococcum	5.3	2.8	0.34	0.99	Hairy	Yellow, purple	Hairy, without hair	Cylindrical	Sub mealy	Yellow
T.dicoccoides	7.8	3.5	0.5	1.2	Without hair	Yellow & green	Without hair	Cylindrical	Sub flinty	Yellow
T.urartu	7.5	4.1	0.5	1.3	Without hair	Yellow & green	Without hair	Cylindrical	Flinty	Yellow & green

cv.: coefficient of variation (1/100)

species (2n=4x=28), *T. dicoccum* (Schrank.) Schübl., *T. dicoccoides* (Körn. ex Aschers. et Graebn.) Thell., *T. turgidum* L. and *T. durum* Desf.; and two hexaploid species (2n=6x=42) *T. aestivum* L. and *T. compactum* Host. The results of morphological surveys are presented in Table 3 and the spike characters of the species recognized are illustrated in Fig. 2a, b.

Based on the results of this study the genus *Triticum* in Iran contains nine species and two subspecies mainly occurring in the Zagros Mountains (Fig. 1). The results of this study are not completely in accordance with those of Boissier (1885) who reported three, Parsa (1954) and Bor (1970) who reported ten, respectively, and Mobayen (1980) who reported nine *Triticum* species in Iran (Table 1). Our results show that the herbarium materials examined belong to six species (Table 2).

Three species (*T. orientale* Percival, *T. polonicum* L. and *T. spelta* L.) recorded by Bor (1970) were not recognized in this study. Bor mentioned only one locality for each of *T. orientale* and *T. polonicum*: "Khorasan, Sykes" and "Azarb., Khoy, Coll. Ign. 358" respectively. In addition, he reported *T. spelta* from Iran based on one doubtful report: "Found in the Bakhtiari country from Shahr-e Kord to

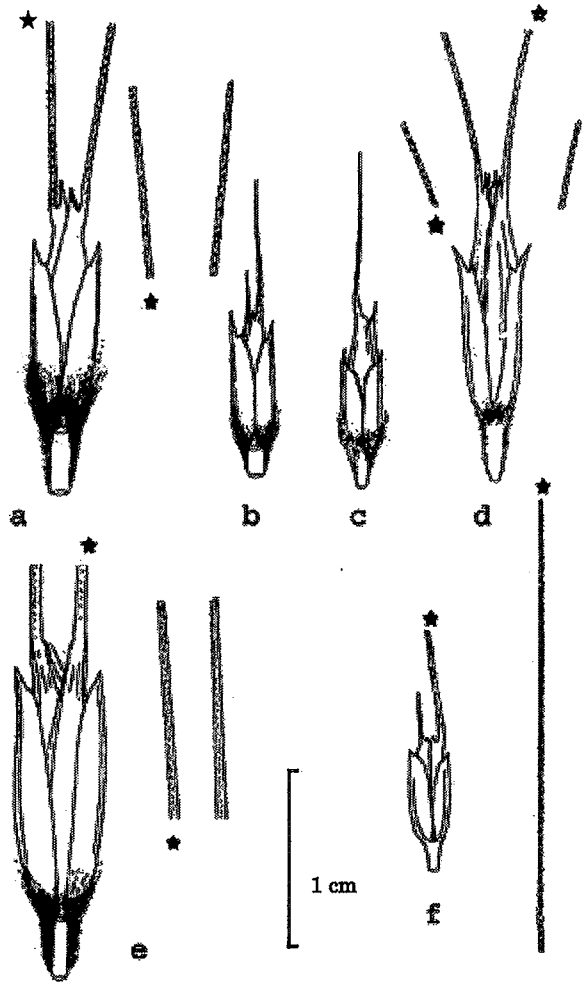

Fig. 2a. The morphology of spikelet in species of *Triticum* L.
a: *T. boeoticum* ssp. *thaoudar* with two equal awn, b: *T. boeoticum* ssp. *boeoticum* with unequal awn, c: *T. monococcum* with one long and one very short awn, d: *T. urartu* with two long equal awn and one short awn in floret, e: *T. dicoccoides* with two awn and long spikelet, f: the spikelet of *T. dicoccum* with two awn,

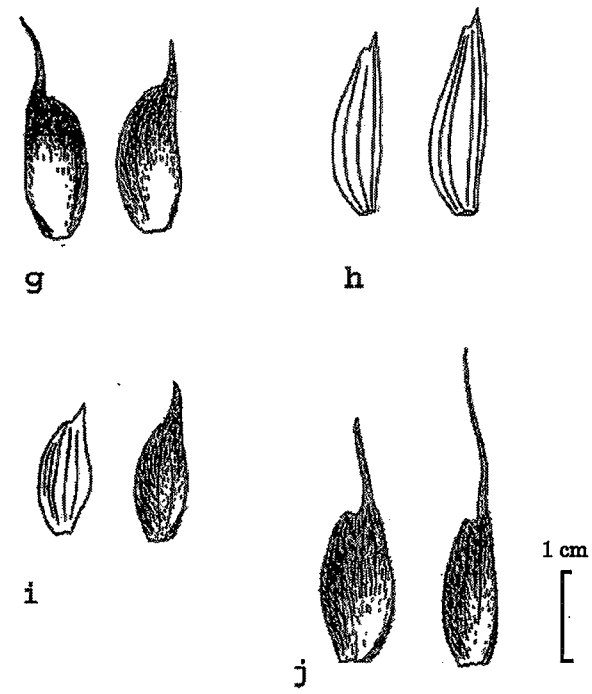

Fig. 2b. The morphology of glume in species of *Triticum* L.
g: *T. turgidum* (keeled from base to apex), h: *T. durum*, i: *T. compactum*, j: *T. aestivum* (keeled in the upper helf only).

Shanseabad and Buldaji, Kuckuck (Wheat Inf Serv No. 9, 1959)". Our expedition in the area did not find this species there; in addition it should be mentioned that the correct name for Shanseabad is Shamsabad, which is a village near Buldaji. Our study revealed two new species, *T. urartu* and *T. compactum* for Iran. We found the latter species as growing in the wheat fields (*T. aestivum*), probably arising as comtaminants of the seeds of *T. aestivum*.

While there is a trend among more recent *Triticum* students to lump the taxa within each ploidy level (see Mac Key 1988 and Van Slageren 1994), the older researchers were more inclined split them (Flaksberger 1935; Schiemann 1948; Jakubziner 1958; Bowden 1959; Morris and Sears 1967). Following the morphological results of this study and also the presence of gene flow barriers among diploid *Triticum* species reported by Filatenko et al. (2001), the diploid species *T. urartu*, *T. monococcum* and *T. boeoticum* are treated as distinct species in this study.

Key to the species of the genus *Triticum* in Iran.

1- Non- free threshing; rachis tough or fragile (broken easily) and ciliate:
  2- Spikelet with 1–2 seeds, narrow and falling; nodes of stem hairy or ciliate, violet or brown:
    3- Lemma of the uppermost floret of each spikelet 1-toothed and awnless.....*T. urartu*
    3- Lemma of all florets untoothed and with 1-2 awns
      4- Lemma with 1 awn .....*T. monococcum*
      4- Lemma with two awns
        5- Lemma with unequal awns
        ..... *T. boeoticum* subsp. *thaoudar*
        5- Lemma with unequal awns.
        .....*T. boeoticum* subsp. *boeoticum*

2- Spikelets with 2-4 seeds, broad and non-falling; nodes of stem ciliate, yellow or straw colored:

6- Rachis tough; lemma of uppermost spikelet usually with 1 awn (rarely with 2 awns) and. that of lowermost spikelet with 1 awn..... ..... *T. dicoccum*

6- Rachis fragile; lemmas always with 2 awns..... ..... *T. dicoccoides*

1- Free threshing; rachis tough and ciliate:

7- Spike quadrate in cross section; spikelets compacted in the spike; nodes of stem glabrous

8- Endosperm flinty; leaf glabrous (rarely hairy); rachis-long ciliate, spike 9.3-21.c ..... *T. durum*

8- Endosperm mealy; leaf hairy; rachis shortly ciliate; spike 10.2-14.7 cm .....*T. compactum*

7- Spike elliptic or ovate in cross section; spikelets more or less loosely arranged in the spike; nodes of stem hairy or ciliate:

9- Spike ovoid, 8.7-23.1 cm; glume keeled from base to apex ..... *T. turgidum*

9- Spike obtriangular, 9.2-25.8 cm; glume keeled in the upper half only ....*T. aestivum*

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References

Boissier PE (1885) Flora Orientalis. Basel, Geneva, Switzerland: H. Georg 5: 672- 673.

Bor NL (1970) Gramineae. In: Rechinger KH (ed) Flora Iranica. Akadem Druck-U. Verlagsanstalt: Graz, Austria, Wien 70: 203- 211.

Bowden WM (1959) The taxonomy and nomenclature of the wheat, barley, ryes and their relatives. Can J Bot 37: 657-684.

Flaksberger CA (1935) Cereals: Wheat. In: Wulf EV (ed) Flora of cultivated plants I Cos Izd Kolkh Sovkh, Moscow and Leningrad (St Petersburg).

Filatenko AA, Grau M, Knupffer H and Hammer K (2001) Discriminative characters of diploid wheat species. In: Hernandez P, Moreno MT, Cubero JI and Martin A (ed) 4th Int Triticeae Symp, Cordoba, Spain.

Gupta PK (1997) Nomenclature in Triticeae with emphasis on D genome diploid species. Wheat Inf Serv 85: 52-55.

Gupta PK and Baum BR (1989) Stable classification and nomenclature in the Triticeae: desirability, limitation and prospects. Euphytica 41: 191-197.

Holmgren PK, Holmgren NH and Barnett LC (1990) Index Herbarium. Part I: The Herbaria of the World. New York: New York Bot Garden.

Jakubziner MM (1958) New wheat species. Proc 1st Int Wheat Genet Symp, Winnipeg,: 207-220.

Lange W and Jochemsen G (1992) Use of gene pools of *Triticum turgidum* and *Aegilops squarrosa* for the breeding of common wheat through chromosome doubled hybrids. Euphytica 59: 213-220.

Mac Key J (1988) A plant breeder's perspective on taxonomy of cultivated plants. Biol Zentralbl 107: 369-379.

Mobayen S (1980) Flora of Iran. Tehran Univ Press.

Morris R and Sears ER (1967) The cytogenetics of wheat and its relatives. In: Quisenberry KS and Reitz LP (ed) Wheat and wheat improvement. Amer Soc Agro, Madison WI: 19-87.

Morrison LA (1993) *Triticum – Aegilops* systematics. In: Damania AB (ed) Biodiversity and wheat improvement. John Willey & Sons, New York.

Morrison LA and Raupp WJ (1999) GrainTax synonym tables project. Wheat Inf Serv 88: 52-56.

Parsa A (1950) Flore de Iran. Ministry of Education, Tehran 139: 807- 815.

Schiemann E (1948) Weizen, Roggen und Gerste: Systematik, Geschichte und Verwendung. G. Fischer Verlag, Jena: 102.

Stearn WT (1992) Botancal Latin. Fourth edition, Timber Press Oregon.

Van Slageren MW (1994) Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. & Spach) Eig (Poaceae). Wageningen Agric Univ: 513.

Vavilov NI (1992) Origin and geography of cultivated Plants. Cambridge Univ Press, Cambridge.

Waines JG and Kimber G (1973) Sattelite number and size in *Triticum monococcum* L. and the evolution of the polyploid wheats. Can J Genet Cytol 15: 117-122.

Yen CJ, Yang L and Yen Y (1997) The history and the correct nomenclature of the D genome diploid species in Triticeae (Poaceae). Wheat Inf Serv 84: 56-59.

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Research article

Potential selection criteria for yield of bread wheat under early and late heat stresses in a dry irrigated environment

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Summary

The predominating high temperatures during early and late stages of wheat in the tropical and subtropical areas are major limitations for its productivity. Searching for relevant selection criteria associated with yield under these areas is of paramount importance. This study was conducted for two seasons at the Gezira Research Farm, Wad Medani, Sudan, using ten bread wheat genotypes and three sowing dates (early, optimum and late). In the second season, high temperatures decreased grain yield by 25% compared with the first season. The differences between sowing dates were clear in the first season for many traits including grain yield. Early and late heat stresses caused reductions of 16 and 32% in grain yield, respectively. Grain yield of the three sowing dates significantly correlated with biomass, grains/m^2, spikes/m^2, grain growth rate, biomass growth rate and vegetative growth rate. In addition, the early sowing grain yield significantly correlated with grains/spike while that of the late sowing correlated with harvest index, thousand kernel weight and grain filling duration. Similar correlations were also found between the heat stress intensity of yield with most of the counterpart traits in the early and late sowings. These results suggest biomass, grains/m^2, spikes/m^2 and vegetative growth rate as selection criteria under early heat stress. Harvest index, thousand kernel weight and grain growth rate could be used as selection criteria under late heat stress.

Key words: bread wheat, heat stress, selection criteria, heat stress intensity.

Introduction

Heat stress is one of the major constraints of wheat (*Triticum aestivum* L.) production in many areas around the world. Heat stress at late growth stages is a problem in 40% of wheat areas in the temperate environments (Reynolds et al. 2001). Moreover, wheat encounters heat stress during the early growth stages in the tropical and subtropical regions (Elahmadi 1996). In the Sudan, a number of environmental stresses affect wheat productivity, but heat stress remains the main constraint and affects the crop at all stages of its development (Ageeb 1994; Elahmadi 1996). Late heat stress is quite common because of the delay in sowing often practiced by farmers due to many reasons (Ibrahim 1996). However, early heat stress is also important because of the great yearly weather fluctuations (Elahmadi 1996).

Wheat grain yield is the product of the two major

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yield components; the number of grains/m^2 and the individual grain weight. When the crop is exposed to high temperatures before anthesis, reduction in grains number occurs via reduction in spike/m^2 and grains/spike (Shpiler and Blum 1991). The reduction in grain weight results from reductions in the grain filling duration and rate (Shpiler and Blum 1991; He and Rajaram 1994). Some studies were able to identify traits that could be used as selection criteria under heat stress conditions. Nonetheless, the uniqueness of the wheat growing environment in the Sudan necessitates the search for relevant selection criteria that might be associated with yield under such environment and accelerate developing heat-tolerant wheat cultivars. In such environment, multiple selection criteria must be applied to predict the crop response to yearly-fluctuating weather. Thus, this experiment was carried out to fulfill the above objectives.

Materials and methods

Eight bread wheat cultivars and two advanced lines were selected to represent different origin and eras of wheat improvement in the Sudan. The cultivars were Beladi, Falchetto, Giza 155, Mexicani, Condor, Debeira, El Nielain and Sasaraib. The two lines, VYT #3 97/98 and VYT #8 97/98, were selected from the national yield trial of the Sudan in 1997/98. The experiment was conducted for two seasons, 1997/98-1998/99 at the Gezira Research Farm (GRF), Agricultural Research Corporation (ARC), Wad Medani, located on the clay plain of the central Sudan (14°24' N, 33° 29' E, 407 m asl). The soils of GRF are cracking heavy clay vertisols, with very low water permeability, pH of 8.5, poor in organic matter (0.5%), deficient in nitrogen (300-400ppm), and low in available phosphorus (4~5 ppm extractable P).

Three sowing dates; early, optimum and late (1st, 3rd week of November and 2nd week of December, respectively) were used during the two seasons. Early sowing date subjects wheat to heat stress during the early stages of the crop, which affect the establishment of the crop and the development of tillers and spikelets. This permits differentiation of genotypes according to their reaction to the early heat stress. The late sowing allows the crop to grow and mature under high temperatures and the response of different genotypes to late heat stress can be assessed.

Seeding at the rate of 120 kg/ha was done manually in plots consisted of six rows 5 m long and 20 cm apart. Fertilization was done using triple superphosphate (43 kg/ha of P_2O_5) prior to planting, while urea was broadcasted before the second irrigation (86 kg/ha of N). Irrigation was carried out at 7-10 days intervals to avoid any water stress. The experiment was arranged in a randomized complete block design with three replications.

Data were collected on date of anthesis, date of maturity, grain filling duration, plant height and number of spikes/m^2. A net area of 3.2 m^2 was hand harvested from the ground level. The harvested material was sun-dried, weighed, threshed and the grains were weighed again to give biomass and grain yield. Grain number/m^2 was calculated as grain yield (g/m^2) divided by individual grain weight. Grains/spike, thousand kernel weight and harvest index were also calculated. Grain growth rate was calculated as grain yield divided by grain fill duration. Biomass growth rate was calculated as biomass divided by days from seedling emergence to maturity, while vegetative growth rate was calculated as (biomass less grain weight) divided by the days from emergence to anthesis.

The heat stress intensity (HSI) was defined by the formula adopted by Shpiler and Blum (1991): HSI = 1– Xh/Xo: where Xh is the grain yield or other traits under early and late sowing conditions and Xo is the grain yield or other traits under optimum sowing condition. Standard statistical analysis of variance was done. Means were compared using Tukey's test. Simple correlations of grain yield with other traits were computed as well as correlations between the HSI of grain yield and other traits.

Results and discussion

Weekly maximum, minimum and mean temperatures during the two cropping seasons are given in Fig. 1 together with the time ranges of anthesis and maturity of the three sowing dates. Temperatures in the second season were 3 to 6°C higher than in the first season. This was clearly affected grain yield and other related traits. Grain yield in the second season was reduced by 25 % compared with the first season. In agreement with Fischer and Maurer (1976), a 1°C rise in temperature reduced grain yield by about 4%.

In the first season, a reduction of 16% was found in grain yield of early sowing compared with the optimum sowing (Table 1). As well as grain yield, biomass, grains/m^2, grain growth rate, biomass growth rate, vegetative growth rate, spikes/m^2, days to anthesis and plant height were all reduced in different degrees by early sowing. On the other hand, grain filling duration was significantly longer in the early sowing. Harvest index, grains/spike, thousand kernel

Table 1. Response of grain yield and other traits of ten bread wheat genotypes to different sowing dates during two seasons; 1997/98 and 1998/99 at GRF

Trait	First season (1997/98)					Second season (1998/99)				
	Sowing dates			% reduction†		Sowing dates			% reduction†	
	Early	Optimum	Late	Early	Late	Early	Optimum	Late	Early	Late
Grain yield (kg/ha)	3276 b‡	3895 a	2630 c	16	32	2517 a	2516 a	2304 b	0	8
Biomass (kg/ha)	8664 b	10075 a	7476 c	14	26	6767 b	7226 a	6192 c	6	14
Harvest index (%)	38.1 a	38.6 a	35.1 b	1	9	37.5 a	34.8 b	37.2 a	-8	-7
Grains/m² (No.)	10445 b	12036 a	8803 c	13	27	8282 b	8818 a	8087 b	6	8
Spikes/m² (No.)	491 b	532 a	477 b	8	10	461 a	451 ab	438 b	-2	3
Grains/spike (No.)	47.0 a	47.7 a	45.4 a	1	5	40.6 a	38.8 b	37.8 b	-5	3
Thousand kernel weight (g)	31.9 ab	32.4 a	30.2 b	2	7	30.6 a	28.6 b	28.6 b	-7	0
Days to anthesis (d)	57 b	59 a	59 a	3	0	57 a	57 a	54 b	0	5
Days to maturity (d)	93 a	93 a	91 b	0	2	95 a	88 b	84 c	-8	5
Grain fill duration (d)	36 a	34 b	32 c	-6	6	38 a	31 b	30 c	-23	3
Plant height (cm)	76 b	78 a	73 c	3	6	72 b	76 a	64 c	5	16
Grain growth rate (kg/ha/d)	92.6 b	114.4 a	81.5 c	19	29	66.5 c	81.3 a	76.2 b	18	6
Biomass growth rate (kg/ha/d)	93.6 b	107.8 a	82.0 c	13	24	71.4 b	82.8 a	73.9 b	14	11
Vegetative growth rate (kg/ha/d)	94.6 b	104.5 a	82.2 c	9	21	74.7 b	83.7 a	72.7 b	11	13

† % reduction from that of the optimum sowing date.

‡ Numbers followed by the same letter(s) in the same row are not significantly different at the 5% level according to Tukey's test

weight and days to maturity remained unaffected by the early heat stress.

The effect of late heat stress was clearly reflected in the great reduction in almost all traits measured (Table 1). Grain yield, biomass, grains/m², spikes/m², grain growth rate, biomass growth rate and vegetative growth rate were more heat sensitive. A reduction of 32% was found in grain yield of the late sowing compared with the optimum sowing. Similar reductions were also found in biomass, grains/m², grain growth rate, biomass growth rate and vegetative growth rate. Reduction in harvest index, spikes/m², thousand kernel weight, days to maturity, grain filling duration and plant height were all significant.

During the second season, the grain yield of the optimum sowing was the same as the early sowing, and only 8% higher than that of the late sowing (Table 1). This was due to that the grain yield of the optimum sowing in the second season was reduced by 35% compared with that of the first season while yields of the early and late sowings were reduced by 23 and 12%, respectively.

Significant reductions in the early sowing biomass, grains/m², grain growth rate, biomass growth rate and vegetative growth rate were observed. On the other hand, harvest index, grains/spike, thousand kernel weight, days to maturity and grain filling duration were significantly better under the early sowing (Table 1). With the late sowing, all traits measured, except harvest index, spikes/m², grains/spike and thousand kernel weight were significantly reduced compared with the optimum sowing. Across the two seasons, biomass, grains/m², grain growth rate, biomass growth rate, vegetative growth rate and plant height were more sensitive to heat stress during early stages of

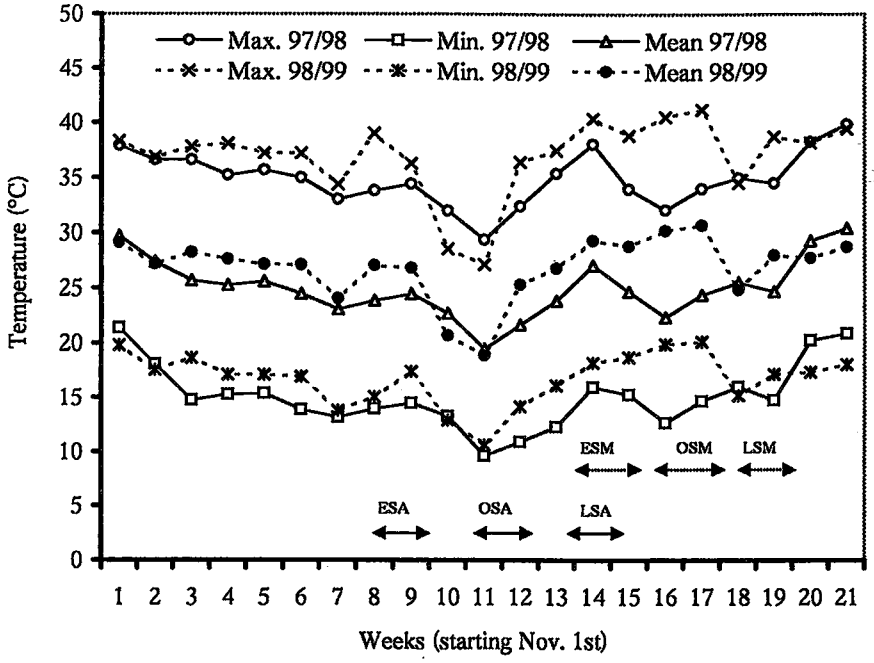

Fig. 1. Weekly max., min. and mean temperatures at GRF during 1997/98 and 98/99 cropping seasons.
Time ranges of the anthesis and maturity are shown. ESA and ESM are early sowing anthesis and maturity, OSA and OSM are optimum sowing anthesis and maturity, LSA and LSM are late sowing anthesis and maturity, respectively.

growth.

High temperatures late in the season were more detrimental to most of the traits during the two seasons. Biomass, grains/m², grain growth rate, biomass growth rate, vegetative growth rate, days to maturity, grain filling duration and plant height were negatively affected during both seasons. Harvest index and thousand kernel weight were significantly lower only in the first season. In the second season, the high temperatures during the grain filling period of the optimum sowing (Fig. 1) negatively affected the two traits. Limitation in the source to meet the demand of high sink might have been important beside the big reduction in the grain filling duration especially when compared with that of the early sowing.

Table 2. Correlation coefficients of grain yield and its heat stress intensity (HSI) of ten genotypes with 13 different traits and their HSI under three sowing dates grown at GRF for two seasons (n = 20). HSI was calculated from the data of the early and late sowings against the data of the optimum sowing

Traits/HSI	Grain yield			Grain yield HSI	
	Early sowing	Optimum sowing	Late sowing	Early sowing	Late sowing
Biomass	0.803***	0.938***	0.740***	0.931***	0.753**
Harvest index	0.402	0.777***	0.662***	0.116	0.858***
Grains/m²	0.762***	0.932***	0.812***	0.801***	0.668*
Spikes/m²	0.635**	0.856***	0.536*	0.313	0.475
Grains/spike	0.477*	0.619**	0.148	0.132	-0.309
Thousand kernel weight	0.387	0.763***	0.602**	-0.062	0.646*
Days to anthesis	-0.139	0.024	-0.047	0.498	-0.078
Days to maturity	-0.187	0.508*	0.210	0.572	-0.017
Grain filling duration	-0.057	0.645**	0.553**	0.297	0.042
Plant height	-0.264	-0.129	-0.180	0.260	-0.172
Grain growth rate	0.901***	0.928***	0.890***	0.898***	0.872***
Biomass growth rate	0.878***	0.961***	0.850***	0.939***	0.720*
Vegetative growth rate	0.736***	0.882***	0.532*	0.835**	0.102

*, **, *** Significant at the 0.05, 0.01 and 0.001 levels, respectively.

Association between grain yield and other traits: Simple correlations were computed between grain yield and other traits of the ten genotypes for each sowing date across the two seasons. Grain yield consistently and significantly correlated with biomass, grains/m^2, spikes/m^2, grain growth rate and biomass growth rate (Table 2). However, the magnitude of the correlation coefficients varied with different sowing dates. Harvest index and thousand kernel weight were significantly correlated with the grain yield of the optimum and late sowings but not with that of the early sowing. In contrast, grains/spike showed significant correlation with the grain yield of the early and optimum sowings but not with that of the late sowing. The above results suggest that biomass, grains/m^2, spikes/m^2, grain growth rate, biomass growth rate and vegetative growth rate are important traits under early heat stress conditions. The importance of biomass and spikes/m^2 confirmed results reported under similar conditions (Ishag and Badredin 1996; Reynolds et al. 1998). On the other hand, harvest index, thousand kernel weight and grain filling duration might be useful predictors of the grain yield for the late sowing date together with biomass, grains/m^2, grain growth rate and biomass growth rate. Many reports found a close association between grain yield and grains/spike and suggested grains/spike as a selection criterion for heat stress tolerance (Shpiler and Blum 1991; He and Rajaram 1994). Under the conditions of the current study, the time when the grains/spike is determined, especially for the late sowing date, occurs usually during the coolest parts of the season.

The association of heat stress intensity of grain yield with that of other traits: The heat stress intensity of yield between the optimum and early sowings was strongly associated with that of biomass, grain/m^2, grain growth rate, biomass growth rate and vegetative growth rate (Table 2). No such associations were found with harvest index, thousand kernel weight and grain filling duration. In the case of late sowing, heat stress intensity of grain yield was associated with that of biomass, grains/m^2, grain growth rate and biomass growth rate but was not strong as in the case of the early sowing except for grain growth rate. On the other hand, stress intensity of harvest index and thousand kernel weight were significantly associated with that of grain yield of the late sowing date.

The strong associations between stress intensity of grain yield with that of biomass, biomass growth rate and vegetative growth rate reinforce the importance of biomass production when early heat stress is concerned. Genotypes that fail to produce sufficient biomass under the early heat stress conditions suffer more than those that are able to keep their biomass production level high. A typical example is the two contrasting genotypes Condor and VYT #3 97/98. The former showed a great reduction in its biomass production and hence low yield, while the latter was able to produce more biomass, and hence more yield, under the early sowing condition (Data not shown). Grains/m^2 also proved to be important trait under both heat stress conditions, but more important under early heat stress conditions. Our results on grain growth rate and grain filling duration demonstrated that although grain filling duration might be important for late sowing, grain growth rate was more closely related with grain yield under all growing conditions, suggesting the importance of this trait under all heat stress conditions (Wardlaw and Moncur 1995; Zahedi et al. 2003). Grains/spike, spikes/m^2 and vegetative growth rate might be useful traits for the early heat stress. On the other hand, harvest index and thousand kernel weight were shown to be more important under late heat stress.

Potential selection criteria: Improving wheat yield under stressed environments is a difficult task compared with favorable environments. Under such condition, multiple selection criteria should be applied to accelerate the development of high yielding and stable genotypes.

Based on the results of associations of yield and its HSI with other related traits, a number of traits can be identified as selection criteria under both early and late heat stress conditions. Biomass, grains/m^2, spikes/m^2 and vegetative growth rate could be used as selection criteria under early heat stress. Harvest index, thousand kernel weight and grain growth rate together with biomass and grains/m^2 could be potential selection criteria under late heat stress condition.

A number of physiological traits were proposed as selection criteria under heat stress conditions (Reynolds et al. 1998, 2001) including canopy temperature depression, stomatal conductance and membrane thermostability. Assessment of wheat genotypes by combining these traits and those identified in this study could considerably improve the efficiency of developing heat tolerant cultivars.

Currently, the performance of promising lines is verified by testing in different locations in all major wheat producing areas in the Sudan. This seems to be useful to study the reaction of those genotypes in a specific environment, but it does not give ideas about how such genotype will react when grown early or late in the season. Ali and Ageeb (1994) mentioned that late planting offers a mean to select for late heat tolerance more efficiently than normal planting, and it has proved to be useful in identifying easily

measurable traits associated with yield under heat stress conditions of Egypt and the Sudan. Tolerance for heat is required at both early and late stages of crop growth in the Sudan due to the short cool period during the season. Therefore, it might be important to conduct trials in different sowing dates at least using the most promising lines in few representative locations so that it can be cost-effective and selecting tolerant cultivars using traits identified in this study.

References

Ageeb, OAA (1994) Agronomic aspects of wheat production in Sudan. In: Saunders DA and Hettel GP (ed) Wheat in heat-stressed environments: Irrigated, dry areas and rice wheat farming systems. CIMMYT, Mexico DF: 67-74.

Ali AMA and Ageeb OAA (1994) Breeding strategy for developing heat-tolerant wheat varieties adapted to Upper Egypt and Sudan. In: Saunders DA and Hettel GP (ed) Wheat in heat-stressed environments: Irrigated, dry areas and rice wheat farming systems. CIMMYT, Mexico DF: 33-39.

Elahmadi AB (1996) Review of wheat breeding in the Sudan. In: Ageeb OA, Elahmadi AB, Solh MB and Saxena MC (ed) Wheat production and improvement in the Sudan. Proc Natl Res Review Workshop, 27-30 August 1995, Wad Medani, Sudan. ICARDA/ Agricultural Research Corporation. ICARDA, Aleppo, Syria: 33-53.

Fischer RA and Maurer RO (1976) Crop temperature modification and yield potential in a dwarf spring wheat. Crop Sci. 16: 855-859.

He Z and Rajaram S (1994) Differential responses of bread wheat characters to high temperature. Euphytica 72: 197-203.

Ibrahim OH (1996) Effect of sowing time on wheat production. In: Ageeb OA, Elahmadi AB, Solh MB and Saxena MC (ed) Wheat production and improvement in the Sudan. Proc Natl Res Review Workshop, 27-30 August 1995, Wad Medani, Sudan. ICARDA/ Agricultural Research Corporation. ICARDA, Aleppo, Syria: 90-98.

Ishag HM and Badreldin AM (1996) Phasic development of spring wheat and stability of yield and its components in hot environments. Field Crops Res. 46:169-176.

Reynolds MP, Singh RP, Ibrahim A, Ageeb OAA, Larque-Saavedra A and Quick JS (1998) Evaluating physiological traits to complement emprical selection for wheat in warm environments. Euphytica 100: 85-94.

Reynolds MP, Nagarajan S, Razzaque MA and Ageeb OAA (2001) Heat stress. In: Reynolds MP, Ortiz-Monasterio JI and McNab A (ed) Application of physiology in wheat breeding. CIMMYT, Mexico DF: 124-135.

Shpiler L and Blum A (1991) Heat tolerance for yield and its components in different wheat cultivars. Euphytica 51: 257-263.

Wardlaw IF and Moncur L (1995) The response of wheat to high temperature following anthesis. 1. The rate and duration of kernel filling. Aust J Plant Physiol. 22: 391-397.

Zahedi M, Sharma R and Jenner CF (2003) Effects of high temperature on grain growth and on the metabolites and enzymes in the starch-synthesis pathway in the grains of two wheat cultivars differing in their responses to temperature. Funct Plant Biology. 30: 291-300.

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Research article

Genetic analysis of economic traits in durum wheat

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Summary

Using 62 elite durum wheat (*Triticum durum*) genotypes, genetic variability, character association and cluster analysis were made to mark the diversity for five important economic traits, namely days to heading, maturity duration, plant height, grain yield per plot and thousand grain weight (TGW). The extent of variability, heritability and genetic advance revealed that enough variability existed for these traits. High estimates of heritability coupled with high genetic advance for thousand grain weight indicate that due to presence of additive gene effects direct selection may be effective. For the traits like grain yield and maturity duration, where high heritability with moderate or low genetic advance was recorded, developing transgressive segregants through hybridization is suggested. Significant and positive correlations between days to heading and maturity duration, thousand grain weight and grain yield, suggest that yield improvement in durum wheat might be possible by emphasizing these traits. This study also provide sound base for selecting better parents from different clusters in order to harness the available diversity. The dendrogram was generated on the basis of Euclidean distances adopting the techniques of complete linkage between genotypes. It was tried to maintain the maximum distances between the clusters to pick most diverse parents. The presence of all three checks (released varieties for different agro-climatic zones) in nearby clusters indicated that geographical distribution has no correspondence to genetic divergence. This also means that there is not much genetic diversity among the released durum varieties for these traits and hence the genotypes from wider clusters must be utilized for yield improvement in durum wheat through hybridization.

Key words: durum wheat, diversity, cluster analysis, dendrogram, Euclidean distances

Introduction

Wheat is the second most important cereal crop after rice in the context to its antiquity and its use as source of food and energy in India as well as in the world. The multifold increase of wheat production in India has mainly been due to introduction and development of semi dwarf varieties. These new varieties have given a cushioning capacity to the sustainable wheat production under vastly varying environments and have proved their worth against various biotic and abiotic stresses. The success of these modern varieties has been sensational, revolutionary and is seen as a result of close collaboration among the Indian wheat breeders and the international agencies particularly CIMMYT and ICARDA. A wealth of wheat genetic resources that has been accumulated in many parts of the world, is available in gene banks, research institutes, laboratories and long term in-situ storage banks. An enormous amount of passport data on these resources have been gathered through collective efforts across the world. In recent past, CIMMYT initiated a data management system on wheat germplasm that can be used by researchers with a unique identifier. In India, Directorate of Wheat Research, Karnal has been engaged in multi-location evaluation of elite germplasm in the form of various nurseries in order

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to provide its excess to wheat researchers and thus enable them to utilize these genetic resources as per their need and priorities. We made an attempt to quantify the genetic variability, heritability, yield architecture and clustering pattern of durum wheat germplasm based on multi-location evaluation, so that the information thus generated may be used accordingly. This paper deals with the results obtained from above study along with the possible suggestive breeding methodology to update the utilization and information generation on some important aspects. This appears to be a optimum assumption for improving yields as well as the wheat production in India.

Materials and methods

A set of 62 elite genotypes of durum wheat (*Triticum durum*) was selected from various national and international sources thereby including material from different origins and agro-ecosystems. Efforts were made to include genotypes from various mega zones considering the characters like duration, height, yield and grain size. The material was planted in a two row plot of 2.5 m length spaced at 23 cm between rows and 10 cm between plants of the row. The experiment was laid out at 12 locations across the country following augmented design wherein the three checks were repeated after every 20th genotype to make statistical analysis precise and useful. At each location similar agronomic practices were followed to raise a good crop. The adjusted means were utilized for estimating genetic variability and other parameters following standard statistical parameters (Johnson et al. 1955).

The observations on five important agronomic traits, namely days to heading, maturity duration, plant height, grain yield per plot and thousand grain weight (TGW) were recorded. Analysis of variance (ANOVA) for all five traits was performed location-wise as well as over pooled data as suggested by Panse and Sukhatme (1967). Analyses then were combined over locations where environment and genotypes were considered and correlations among traits were computed over environments. The pooled data were subjected to cluster analysis as well as to make dendrogram based on the inter-varietal distances (Singh 1994) and accordingly the 62 genotypes were grouped into five clusters considering all the five traits under study. The distances are Euclidean but the pattern is similar to that computed following Mahalanobis distances, however, the dendrogram of which are not shown and discussed here (Mahalanobis 1936; Lee and Kaltsikes 1973). The plotting of linkage distances across steps following Euclidean distances has been made as per the latest available statistical software (Statistica).

Results and discussion

The results of present study revealed that genotypes differed significantly for all the characters except grain yield as indicated by ANOVA for individual traits (Table 1). The observations showed that genotypic variability was high and that prompted and provided us the base to go for further analyses. The estimates of variability parameters for all five traits were worked out and are also presented in Table 1. Mean performance of 62 genotypes for five characters revealed that days to heading varied between 72-86 with an average of 80.14 days having 3.35 critical difference (CD). Similarly, the maturity duration of material ranged between 117-126 days with mean maturity duration of 121 days. The average plant

Table 1. Genetic parameters of five characters in durum wheat

Character	F Value	Mean	Variance		Variability (%)		h^2	GA
			δ^2_P	δ^2_G	PCV	GCV	(%)	(%)
Days to heading	5.72**	80.14	56.70	49.98	9.39	8.82	88.10	16.42
Maturity duration	2.88*	120.94	31.85	24.10	4.67	4.05	75.67	9.79
Plant height	3.30*	81.45	59.26	51.92	9.45	8.85	87.61	16.65
Grain yield	1.47	455.51	298.11	230.37	3.79	3.34	77.28	30.98
Thousand grain weight	4.91**	39.70	47.09	44.95	13.11	12.58	95.45	16.87

*, ** Significant at the 5% and 1% level, respectively.

δ^2_P: Phenotypic variance, δ^2_G: Genotypic variance, PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, h^2 (%): Heritability (broad sense), GA (%): Genetic advance

height was 81 cm whereas the average plot yield was recorded as 455 g. In general the material under study had a mean of 40 g thousand grain weight over locations.

The variance {genotypic and phenotypic and percent variability along with heritability (broad sense)} estimates were calculated (Table1). The genotypic coefficient of variation was moderate for most of the traits except thousand grain weight, which otherwise is also considered as a highly heritable character. The highest estimates of heritability were recorded for thousand grain weight followed by days to heading and plant height. Heritability estimates along with the genetic advance are very useful in predicting the gain under selection instead of heritability alone (Singh and Narayana 1993). High heritability coupled with high genetic advance and high coefficient of variability for thousand grain weight and days to heading. Whereas, high estimates of genetic advance and variance for grain yield per plot indicated that due to additive gene effects direct selection may be effective in this character. However, in case of characters showing high heritability but moderate or low genetic advance, which may be due to non-additive gene action and presence of GxE interaction, simple selection may not be rewarding (Singh et al. 2003, Sharma and Sain 2003). In such cases breeders can go for selections over locations following hybridization and selecting desirable transgressive segregants (Allard 1960; Kumar et al. 2002).

The correlation coefficients (genotypic) among five

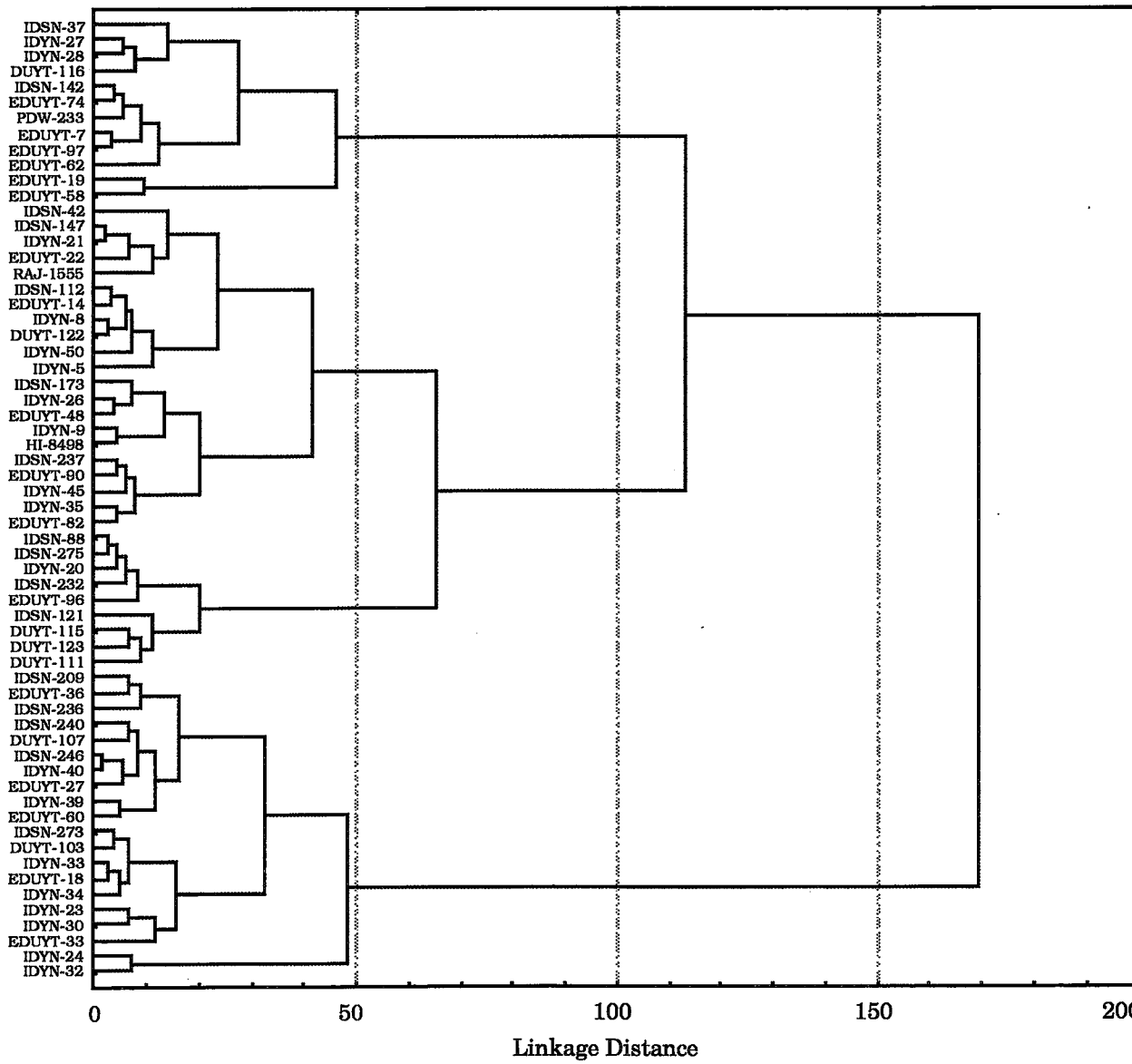

Fig. 1. Dendrogram using Euclidean distances derived from five traits of 62 durum wheat genotypes.

Table 2. Correlation coefficients among five traits in durum wheat

	Maturity duration	Plant height	Grain yield	Thousand grain weight
Days to heading	0.948**	0.209	–0.833**	–0.440*
Maturity		0.024	–0.699**	–0.407*
Plant height			0.345	0.375
Grain yield				0.671**

*, ** Significant at the 5% and 1% level, respectively.

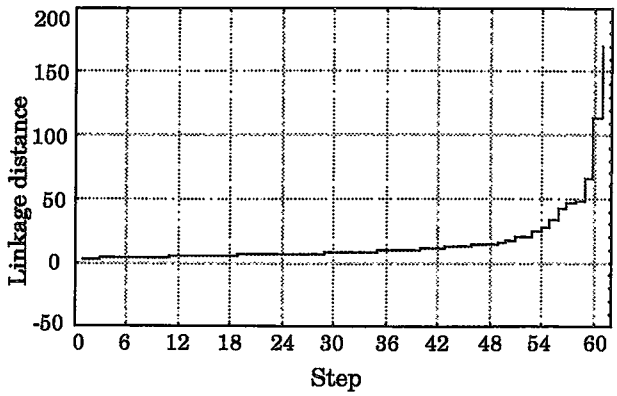

Fig. 2. Plot of linkage distances across steps Euclidean distances.

traits were also worked out to see the association between these traits and are given here in Table 2. Significant and positive correlations were observed between days to heading and maturity duration, thousand grain weight and grain yield, whereas, days to heading and maturity duration showed significant but negative correlations with grain yield. Similarly, thousand grain weight also had significant but negative correlation with heading and maturity duration. The negative association between days to heading, maturity duration with grain yield and thousand grain weight may be due to late heat stress affecting grain development and then ultimately to the yield. This was also evident with moderate mean of thousand grain weight in general. However, the significant and positive association between grain yield and thousand grain weight indicated the direct contribution of grain size to total grain yield. We assume that in durum wheat thousand grain weight has comparatively higher contribution to the yield as compared to bread wheat.

Dendrogram of 62 genotypes was generated on the basis of Euclidean distances created by making use of five parameters and adopting the technique of complete linkage between the genotypes (Statistica software). The critical perusal of the dendrogram reveals that up to the stage 5 the linkage distances between the clusters was appreciable and have little variation thereafter (Fig 1). Plotting of linkage distances across steps following Euclidean distances

Table 3. Cluster-wise distribution of 62 durum wheat genotypes including three checks

Cluster	Number of genotypes	Genotypes included in each cluster
I	9	IDSN 88, IDSN 121, IDSN 232, IDSN 275, IDYN 20, EDUYT 115, EDUYT 96, EDUYT 111, EDUYT 123
II	12	IDSN 42, IDSN 112, IDSN 147, IDYN 5, IDYN 8, IDYN 21, IDYN 45, IDYN 50, EDUYT 14, EDUYT 22, EDUYT 122, RAJ 1555
III	15	IDSN 142, IDSN 173, IDSN 237, IDYN 9, PDW 233, IDYN 26, IDYN 35, EDUYT 7, HI 8498, EDUYT 48, EDUYT 62, EDUYT 74, EDUYT 82, EDUYT 90, EDUYT 97
IV	6	IDSN 37, IDYN 27, IDYN 28, EDUYT 19, EDUYT 58, EDUYT 116,
V	20	IDSN 209, IDSN 236, IDSN 240, IDSN 246, IDSN 273, IDYN 23, IDYN 24, , IDYN 30, IDYN 32, IDYN 33, IDYN 34, IDYN 39, IDYN 40, EDUYT 18, EDUYT 27, EDUYT 33, EDUYT 36, EDUYT 60, EDUYT 103, EDUYT 107
Total	62	

IDSN: International durum wheat screening nursery, EDUYT: Elite durum wheat un-replicated yield trial, IDYN: International durum wheat yield nursery, The three checks namely RAJ 1555, PDW 233 and HI 8498 used, are the released Indian durum wheat varieties.

as presented in Fig. 2 also suggested that up to about 55 steps only 25 % of the total linkage distance has been accounted whereas 75 % of the distance was accounted after step 55 leading to about 5 clusters (Fig. 2). This suggests that the restricting the grouping of genotypes into five clusters will have sufficient distance between the clusters and thus the 62 genotypes were grouped into five clusters. The maximum number of genotypes were in cluster V (20 genotypes) while cluster IV had only six genotypes (Table 3). We noticed that out of three checks which were the released varieties for north west and central India, two (PDW 233 and HI 8498) were in cluster III and though the third check (Raj 1555) was in cluster II, but this being the last entry in this cluster could also have been in cluster III, if we slightly increase the Tocher's value. This indicates that in the genotypes under study enough variability exists, which is more than that in already existing released varieties and thus the same can be utilized for hybridization for obtaining the transgressive segregants for desired traits (Deshmukh et al. 1999, Raut et al. 1985). The future yield improvement in durum wheat might be made or achieved by utilizing the information and material developed from the present study.

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References

Allard, RW (1960) Principles of plant breeding. John Wiley and Sons Inc, New York.

Deshmukh PB, Atale SB, Pandey MK, Vitkare DG and Golhar SR (1999) Genetic divergence in durum wheat. Crop Improv 26: 95-98.

Johnson HW, Robinson HF and Comstock RE (1955) Estimation of genetic and environmental variability in soybeans. Agron J 47: 314-318.

Kumar RR, Singh Meenakshi and Singh SP (2002) Genetic variability and heritability studies in *Cuphea procumbans*. Ind J Plant Genet Resour 15(1): 40-45.

Lee J and Kaltsikes PJ (1973) The application of Mahalanobis's generalized distance to measure genetic divergence in durum wheat. Euphytica 22: 124-131.

Mahalanobis PC (1936) On the generalized distance in statistics. Proc Nat Acad Sci (India), 12: 49-55.

Panse VG and Sukhatme PV (1967) Statistical methods for agricultural workers. Ind Council of Agric Res, New Delhi.

Raut VM, Rao VSP, Patil VP and Deodikar GB (1985) Genetic divergence in *Triticum durum*. Ind J Genet 45: 141-151.

Sharma SN and Sain RS (2003) Genetic architecture of grain weight in durum wheat under normal and late sown environments. Wheat Inf Serv 96: 28-32.

Singh P and Narayana SS (1993) Biometrical techniques in plant breeding. Kalyani Publishers, New Delhi.

Singh PK (1994) Genetic diversity in durum wheat (*Triticum durum* Desf.). Ann Agric Res 15: 418-422.

Singh R, Kharb RPS and Singh Vikram (2003) Genetic divergence study in durum wheat based on seed vigor parameters. Wheat Inf Serv 96: 20-22.

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Research article

Studies on breeding of dominant nuclear dwarf male sterile lines with a blue seed marker in common wheat

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Summary

The genotype 89-2343 of durum wheat with *Ms2* and a blue seed marker was crossed and backcrossed with a common wheat genotype 7739-3 to produce male fertile plants with blue seeds (MFP-BS). To combine the blue seed marker, dwarf male sterile plants carrying *Rht10* and *Ms2* were fertilized with pollen from selected MFP-BS. At last, the combination of blue seed marker, *Ms2* and *Rht10* was successfully produced. The segregation ratio of male sterility, seed color as well as chromosome configurations of the combinations suggested that the blue seed marker, *Ms2* and *Rht10* were located on the same chromosome. Cytological analysis indicated that the male sterile wheat line with a blue seed marker was 43 in chromosome number, with an additional chromosome. The transmission rate for blue seed male sterile plants was 22.1%. In addition, the potential value for blue marker sterile lines in wheat breeding and hybrid production is discussed.

Key words: *Triticum aestivum*, blue seed marker, dwarf male sterile line, cytological analysis, hybrid production

Introduction

China Taigu wheat is one kind of nuclear male sterility germplasm. The fertility of Taigu wheat is controlled by a single dominant nuclear male sterility gene *Ms2* (Deng and Gao 1980). All of the male sterile plants are heterozygous (*Ms2ms2*). So, hybrid seeds from male sterile plants always produced 50% fertile and 50% male sterile plants. (Deng and Gao 1980; Liu et al. 1986). After many efforts, the combination of the dominant dwarf gene *Rht10* and the male sterility gene *Ms2* was established, which makes it possible to discriminate fertile plants from male sterile lines during heading stage (Liu and Yang 1991). At the same time, the dwarf male sterile line still could not be widely used for hybrid seed production because the fertile plants have to be removed before flowering, which increased seed production cost. If hybrid seeds of fertile plants could be discriminated from those of male sterile plants, the dominant male sterile line could be used for multiplying male sterile seeds by making crosses between male sterile and fertile plants with the same genetic background and to produce hybrid seeds by crossing male sterile plants with normal varieties or elite lines. After screening out the sterile seeds, the hybrid seeds left could be used for yield testing or for production.

It has been proved that the blue aleurone layer of wheat seed is controlled by a dominant gene located on the chromosome 4E of *Elytrigia elongatum* (Li et al. 1982). The chromosome 4E of *Elytrigia elongatum* has been successfully added to common wheat and a 4E addition line (2n=42W+4E') with blue seeds has been developed (Sun and Li 1985; Shen et al. 1991). Based on this strategy, we are planning to breed for male sterile wheat lines using blue seed color as selection makers to distinguish fertile seeds from male sterile seeds. It has been established that the dominant gene *Ms2* controls the nuclear male sterility of Taigu wheat located on chromosome 4D (Liu et al. 1986). Therefore, the possibility exists to combine these two genes by pairing and segmental exchange between

partially homologous chromosomes (Sear 1972; Kaul 1987). In this way, a male sterile durum wheat line with a blue seed marker has been bred, whose chromosome constitution is AABB+4D(*Ms2*)/4E (Tian 1991; Zhang 2001). In this paper, we report the breeding of wheat combination lines of *Rht10*, *Ms2* and a blue seed marker. The chromosome structures of male fertile plants and sterile plants, as well as the inheritance ratio of male sterile plants to fertile plants, have been investigated.

Materials and methods

A nuclear male sterile durum wheat line 89-2343 (2n=4x=AABB+4D(*Ms2*)/4E) with a dominant nuclear male sterile gene *Ms2*, was crossed with a wheat line 7739-3 of *Triticum aestivum* L. (2n=6x=42, AABBDD) to produce progenies. Then, another common wheat 4325 of *Tritium aestivum* L. (2n=6x=42, AABBDD (*Ms2ms2Rht10rht10*)) with dwarf male sterile and white seed, was crossed with the fertile plants having blue seed marker from progenies of 89-2343/7739-3 to screen out the common wheat combinations of dominant nuclear male sterile gene *Ms2,Rht10* and a blue seed marker.

The segregation ratios for blue seed male sterile plants and white seed male fertile plants were tested on 13 new male sterile lines, which were derived from the combinations of dominant nuclear dwarf male sterile wheat line with blue seed marker. Cytological check by investigating the root tips and pollen mother cells was made between dwarf male sterile plants with blue seeds and fertile plants with white color.

Hybrid seeds in the same spike generally displayed two colors, i.e. blue and white. In the next season, blue and white seeds harvested from the same spike were planted separately in the field.

Results

Breeding the combinations of dominant nuclear male sterile gene *Ms2*, dwarf gene *Rht10* and a blue seed marker: In order to transmit the addition chromosome 4D(*Ms2*)/4E from durum wheat 89-2343 into common wheat, the cross between durum wheat 89-2343 and hexaploid wheat 7739-3 was made. In this cross, many male-fertile plants with blue seeds (MFP-BS) were produced. The progenies of these plants were all male fertile and their grains were blue. Progenies of most hybrids produced 50% male fertile and 50% male

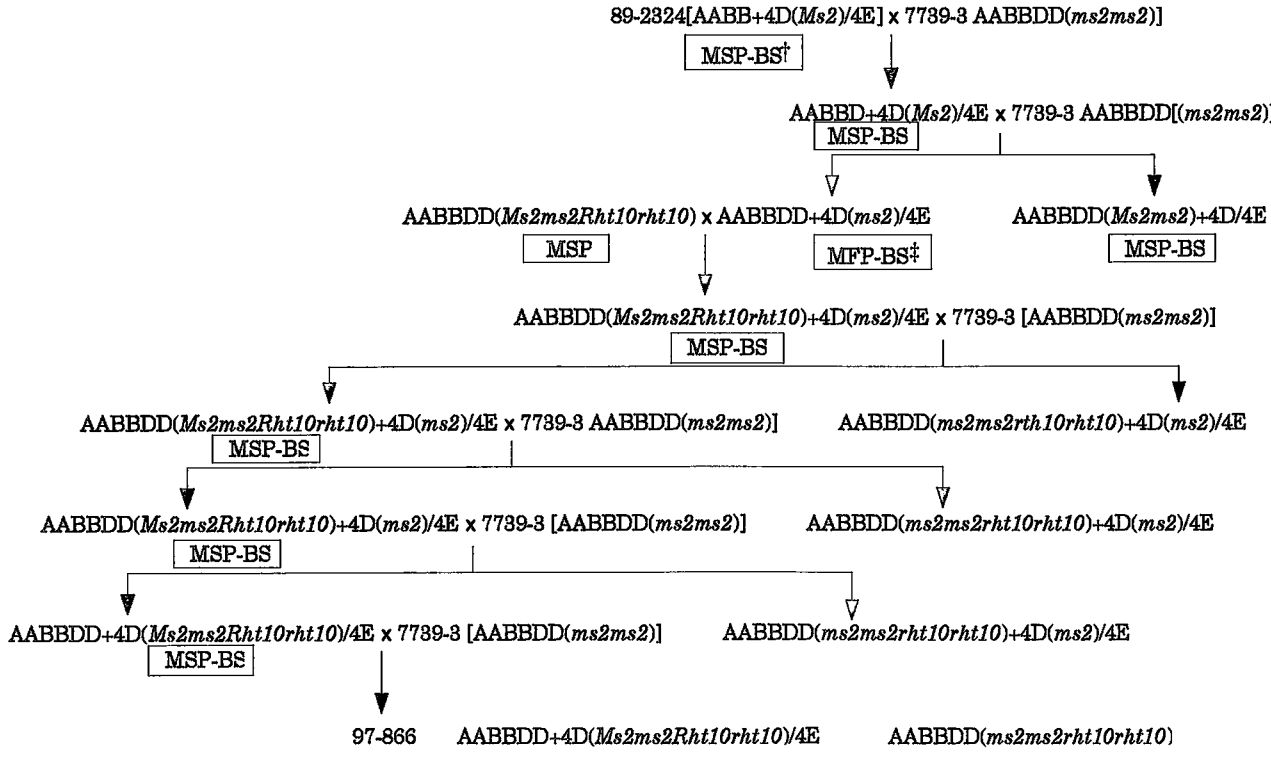

Fig. 1. Diagram of developing male sterile line 97-866 with a blue seed marker in common wheat.
†MSP-BS represents male sterile plants from blue seeds,. ‡MFP-BS represents male fertile plants from blue seeds.

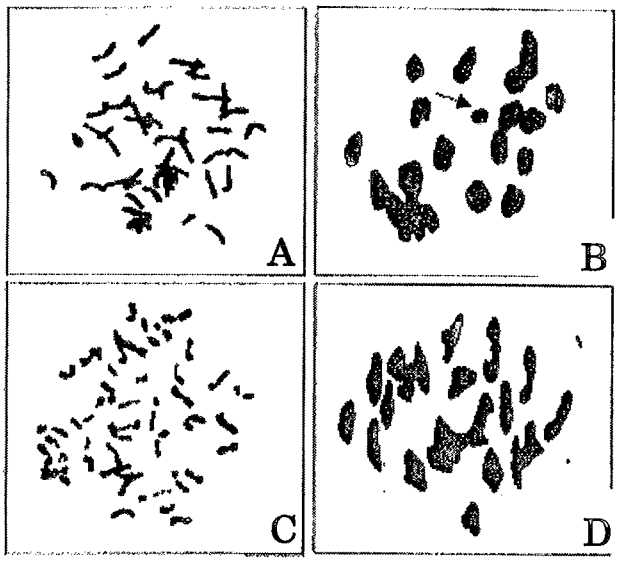

Fig. 2. Cytological analysis of dwarf male sterile plants with blue seeds and fertile plants with white seeds in 97-866.
A: chromosome number (2n=43) in root tip cells from blue seed in 97-866, B: chromosome configuration (21 "+1') at metaphase I in pollen mother cells of male sterile plant from blue seed in 97-866, arrow indicates univalent, C: chromosome number (2n=42) in root tip cells from white seed in 97-866, D: chromosome configuration (21") at metaphase I in pollen mother cells of fertile plant from white seed in 97-866.

sterile plants with blue seed (MSP-BS). This suggested that a segment exchange must have taken place between the additional chromosome 4D(*Ms2*)/4E and probably 4D(*ms2*). The MFP-BS may have AABBDD (*ms2ms2*)+ 4D(*ms2*)/4E and the MSP-BS may be AABBDD (*Ms2ms2*)+4D(*ms2*)/4E.

To combine the blue seed marker with *Rht10* and *Ms2*, the dwarf male sterile plants were fertilized with the pollen from selected MFP-BS. Translocation between 4D(*Ms2ms2Rht10rht10*) and 4D(*ms2*)/4E was expected but nothing happened in the first three years.

Among the 10268 spike lines tested from 1993 to 1997, one wheat line, 97-866, was finally found to have combined *Rht10*, *Ms2* and blue seed marker (Fig. 1). Characteristics of the combinations of *Ms2,Rht10* and a blue seed marker: The characteristics of combinations of *Ms2,Rht10* and a blue seed marker were very similar to those (about 55cm height, loosen in individual plant, blue in grain color and enriched seeds) of the dwarf male sterile parents 4325. Besides, the heading date was 1 to 2 days later than that of their recurrent parent. Stigma was full fertile and had good opening status during flowering. Anther was shriveled, gray and without pollen. Other traits were similar to those of their recurrent parents.
Cytological analysis: The chromosome number in root tips of the combination wheat line 97-866 (based on 10 plants) of *Ms2,Rht10* and a blue seed marker, was 2n=43 (Fig. 2A), but that of male fertile line with white seed was 2n=42 (Fig. 2C). At metaphase I of meiosis in the pollen mother cells, chromosome configuration of combination wheat line of *Ms2,Rht10* and a blue seed marker was 21"+1' (Fig. 2B), whereas, that of male-fertile line with white seed was 21" (Fig. 2D). This suggests that the combination wheat line 97-866 is still a monosomic addition line. All of the plants with blue seed marker were male sterile and short; the white seed plants were male-fertile and tall when planted in the field.
Inheritance analysis: Experiential data of 97-866 from 1998 to 2000 indicated that the transmission rate of short sterile-plant with blue seed marker was 22.1% (21.2~23.5%), and that of white male-fertile plants was 77.7% (Table 1).

13 new sterile wheat combinations of *Rht10, Ms2* and blue seed marker were derived from the crosses among different common wheat background with dwarf male sterile wheat line 97-866. Experimental data from the 18642 spike lines of thirteen dominant nuclear male sterile lines with blue seed marker were obtained in consecutive 3 years (2001~2003). The variance analysis and multiple comparison results suggested that the transmission ratio for short sterile plant with blue marker was still stable at a mean of 22.1% (14.6~48.2%) (Table 2). This result suggested

Table 1. Segregation ratio between seed color and male sterility in 97-866

Year	Blue short sterile	White tall fertile	White short sterile	Blue tall fertile	Blue short sterile (%)
1998	347	1291	2	0	21.2
1999	272	884	2	0	23.5
2000	315	1135	1	1	21.7
Mean					22.1

Table 2. Genotypes and stability analysis for the transfer percentage of dwarf male sterile wheat with a blue seed marker (DMS-BSM)

Genotype	Transfer percentage (%)	Significance of difference		Deviation among years	
		5%	1%	SD	CV%
477A	48.2	a	A	2.12	4.4
455A	28.4	b	B	1.13	4.0
411A	24.5	c	C	1.51	6.2
391A	24.0	cd	C	1.41	5.8
395A	23.3	cd	CD	0.56	2.4
477A	23.1	cd	CD	0.14	0.6
421A	22.5	cd	CD	1.20	5.3
417A	21.7	d	CD	0.35	1.6
393A	21.6	d	CD	0.42	1.9
415A	20.4	de	D	1.84	9.0
413A	20.3	de	D	0.78	3.8
387A	19.2	e	D	0.42	2.2
384A	14.6	f	E	0.28	1.9
Mean	22.1				

that the transmission ratio for additional chromosome 4D/4E was very similar during different years but dissimilar significantly among recurrent parent. In most recurrent parents, the transmission ratio for additional chromosome 4D/4E was about 20%. To our interest, the transmission ratio for dwarf male sterile plant with a blue seed marker in genotype 477A is up to 48.2%.

Discussion

Male sterile plants from the hybrid seeds of male sterile plants were always heterozygous (*Ms2ms2*) for the dominant sterility gene, and fertile plants from the hybrid seeds were homozygous (*ms2ms2*) for the recessive alleles. If male sterile seeds could be distinguished from fertile seeds in a simple way, it would be more convenient to use dominant male sterility in hybrid seed production. Aneuploids in different common wheat background were produced based on the durum wheat addition line 89-2343 (Tian 1991). After extensive backcrosses, a female gamete with a translocation segment containing *Ms2ms2Rht10rht10* in 4D/4E was fertilized. It was finally developed into a combination line with blue seed marker, *Rht10* and *Ms2*. Thus, the fertility of plants could be easily identified according to their seeds color. Meanwhile, transmission rate of additional chromosome 4D/4E was stable during different years. So, female plants from blue seeds will display uniform male sterility, and recurrent selection could be conveniently carried out in wheat breeding program.

Blue seeds, short plants and shriveled anthers controlled by the blue seed genes, *Rht10* and *Ms2* respectively, are easily identified in laboratory and field. Nearly all modern wheat varieties and elite lines could be transferred into the blue marker male sterile lines through backcrossing. When blue marker male sterile lines are crossed with normal varieties, about 80% of the hybrid seeds with a white color will generate normal fertile plants. The fertility of these plants is not affected by restoring gene and so the possibility for breeding new crosses or hybrids is increased. Because the additional chromosome usually has lower transmission ratio in most recurrent parents, so the propagation coefficient of blue male sterile seeds was very low. If the blue kernel gene could be transferred to chromosome 4D, a blue marker male sterile line with 21 pairs of chromosome, would be more effective and stable than addition lines. In this study, it is very interesting that the transmission ratio for blue seed marker, *Rht10* and *Ms2* in genotype 477A was nearly 50% in three seasons. It is still not clear whether the blue seed marker has been transferred to chromosome 4D or not.

References

Deng JY and Gao ZL (1980) The use of a dominant male sterile gene in wheat breeding. Acta Agron Sinica 6: 85-98.

Kaul MLH (1987) Gene-cytoplasmic male sterility. Monographs on Theor etcal Applied Genetics: 96-151.

Li ZS, Mu L and Jiang H (1982) A study on blue-grained monosomic wheat. Acta Agron Sinica 9: 431-439.
Liu B, Yang L and Deng JY (1986) A dominant gene for male sterility in wheat. Plant Breed 97: 204-209.
Liu B and Yang L (1991) Development of dwarf abortion wheat and its prospect in practice. Chinese Sci Bul 3: 306-308.
Sears ER (1972) Chromosome engineering in wheat. Wheat Sci Today Tomorrow 4: 23-28
Shen J, Deng JY and Fan L (1991) A preliminary study on marking blue grain character on Taigu dominant male sterile wheat. Acta Agron Sinica 17: 387-391.
Sun S and Li S (1985) Study on adding a blue grain marker for Taigu nucleus male sterility. Shanxi Agric Sci 1: 24-25.
Tian N (1991) Preliminary study of a durum wheat with additional chromosome. Shanxi Agric Sci 2: 32-34.
Zhang ZB (2001) Wheat Genetics. Chinese Agric Press: 263-265

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Research article

Optimization of tissue culture procedures for the transformation of Chinese elite spring wheat (*Triticum aestivum* L.) varieties via particle bombardment

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Summary

Some factors of tissue culture affecting the transformation of Chinese elite spring wheat (*Triticum aestivum* L.) varieties via particle bombardment were investigated. Among three explant resources (i.e. immature embryos, mature embryos and immature inflorescences), immature embryos were superior to mature embryos and immature inflorescences. Of the 6 callus induction media, the average frequency of callus induction on MS_2 medium was higher than that of other media. Among the 8 Chinese spring wheat genotypes, Chuannong 16 had the highest callus induction frequency, while Chuanmai 32 had the lowest callus induction frequency. For genotype-medium combinations, the immature embryos of Chuannong 16 cultured on MS_2 had the highest frequency of callus induction (99.3%). Comparison of the differentiation frequencies of media MS_{10} and $MS_{10\text{-}1}$, it indicated that MS_{10} was superior to $MS_{10\text{-}1}$. There were 5.6% calli derived from immature embryo transient expressed the blue spot of *gus* gene. These results suggested that the optimization of tissue culture procedures were available for the genetic transformation of Chinese elite spring wheat via particle bombardment.

Key words: wheat, tissue culture, transient expression, microprojectile bombardment

Introduction

Wheat (*Triticum aestivum* L.) is one of the world's most important crops with an annual production exceeding 570 million metric tons (FAO 2003). It is one of the most abundant sources of energy and proteins for mankind. Hence, wheat is a primary target for improvement of agronomic characteristics via biotechnology and genetic engineering (Lazzeri et al. 1997). However, the transgenic wheat plants were not obtained until 1992. Vasil et al. (1992) reported the first successful wheat transformation with a selectable marker gene *bar* and a reporter gene GUS by particle bombardment. Over the last 10 years, wheat genetic transformations via particle bombardment were widely applied for improving its end-used quality and resistance to abiotic/biotic stresses (Altpeter et al. 1996; Blechl and Anderson 1996; Barro et al. 1997; Bedö et al. 1998; Chen et al. 1998; Leckband and Lörz 1998; McIntosh 1998; Vasil et al. 1998; Rasco-Gaunt et al. 1999).

The widespread application of this method is still limited by relatively low and erratic stable transformation efficiencies. The efficiency of stable transformation is strongly dependent on wheat tissue culture and transformation procedures (Alpeter at al. 1996; Hess and Carman 1998; Iser et al. 1999; Rasco-Gaunt et al. 1999, 2001). A few agronomically less desirable model genotypes with good response in tissue culture and transformation, such as Bobwhite and Florida, were generally used in wheat transformation (Rasco-Gaunt et al. 2001). The application of this technology directly to elite wheat cultivars is

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preferable for the breeding programs, while it is not always possible due to the variability in regeneration and frequency rates of transformation between varieties (Iser et al. 1999). Thus, it is essential to investigate the transformation frequencies among elite wheat varieties, and to optimize the procedures for their genetic transformation. The variations of transformation frequency among European and Japanese elite wheat verities had been evaluated (Takumi and Shimada 1997; Iser at al. 1999), and the particle bombardment parameters and procedures for European elite wheat verities had been reported (Rasco-Gaunt et al. 1999, 2001).

In China, wheat, rice and corn are the most important cereal crops. Many elite spring wheat cultivars have been widely used in the southern China. Till date, the informations about the transformation of Chinese elite spring wheat cultivars are not available. The objective of this paper is to optimize the tissue culture procedures of particle bombardment transformation for Chinese elite spring wheat cultivars.

Materials and methods

Plant materials: The mature embryos, immature embryos and inflorescences of eight Chinese spring wheat cultivars or lines were used to evaluate the competence of callus initiation. All materials were randomly planted in experimental field in 2002. Chuannong 16, Chuanyu 16, Chuanmai 32, Chuanyu 12 and 80-8 are elite wheat cultivars, and the remains are excellent breeding lines.

The immature inflorescences with 1.0 to 2.0 cm and the early-medium milk-stage young grains 12-16 days after anthesis were harvested. Mature and young grains, and immature inflorescences were surface-sterilized firstly in 70% ethanol for 1 min, then 10 min in 0.1% mercuric chloride ($HgCl_2$), and followed by three 5-min in sterile distilled water. Mature and immature embryos were aseptically dissected from mature and young grains, respectively, and then placed on media with the embryo-axis facing upward. Immature inflorescences were cut into small pieces of 1-2 mm and placed on media. The explants were cultured in dark at 25°C about 10 days until the calli grow to 10 mm, and then transferred to differentiation medium at 26°C, 16-h photoperiod to induce plant regeneration.

Culture media: The medium compositions were listed in Table 1. Among these, 6 media (No.1 to No.6) were used for callus induction from immature embryo. MS_9 was used for callus maintenance. Two media with different content of auxins, MS_{10} and $MS_{10\text{-}1}$, were used in plantlet differentiation and regeneration. MS_{11} and $MS_{11\text{-}1}$ with different concentration of phytohormone 1-naphthylacetic acid (NAA) were used to induce the root initiation. All media were autoclaved for 20 min at 121°C and 0.11 MPa.

Plasmids construct: The transformation vector pDM803 was used for microprojectile bombardment. The schematic structure of pDM803 was shown in Fig.1. pDM803 contains a chimaeric *bar* gene from *Streptomyces hygroscopicus* (Thompson et al. 1987) under control of the maize *Ubi1* promoter

Table 1. Summary of medium composition

No.	Medium	Composition
1	$N6_0$	N6 + 2 mg/l 2, 4-D + 1 mg/l KT + 100mg/l VC + 6% sucrose + 8 g/l agar, PH5.8
2	$N6_1$	N6 + 2 mg/l 2, 4-D + 1 mg/l KT + 500 mg/l pro + 200 mg/l CH + 100 mg/l VC + 6% sucrose + 8 g/l agar + 200 mg/l asparagine + 200 mg/l Gln, PH5.8
3	MS_0	MS + 2 mg/l 2, 4-D + 1 mg/l KT + 100 mg/l VC + 6% sucrose + 8 g/l agar, PH5.8
4	MS_1	MS + 2 mg/l 2, 4-D + 1 mg/l KT + 500 mg/l pro + 200 mg/l CH + 100 mg/l VC + 6% sucrose + 8 g/l agar + 200 mg/l asparagine + 200 mg/l Gln, PH5.8
5	MS_2	MS macro salts (2X) + MS + 150 mg/l l-asparagine + 100 mg/l VC + 6% sucrose + 8 g/l agar + 2 mg/l 2, 4-D, PH5.8
6	MS_3	MS + 500 mg/l CH + 150 mg/l asparagine + 100 mg/l VC + 60g/l sucrose + 2 mg/l 2, 4-D + 0.4 mg/l KT + 8g/l agar, PH5.8
7	MS_9	MS + 1 mg/l 2, 4-D + 100 mg/l VC +3% sucrose + 8g/l agar, PH5.8
8	MS_{10}	MS + 2 mg/l 6-BA + 0.5 mg/l IBA + 3% sucrose + 8 g/l agar, PH5.8
9	$MS_{10\text{-}1}$	MS + 2 mg/l NAA + 0.5 mg/l KT + 3% sucrose + 8 g/l agar, PH5.8
10	MS_{11}	1/2MS + 1 mg/l NAA + 0.5 mg/l IBA + 3% sucrose + 7 g/l agar, PH5.8
11	$MS_{11\text{-}1}$	1/2MS + 2 mg/l NAA + 0.5 mg/l IBA + 3% sucrose + 7 g/l agar, PH5.8

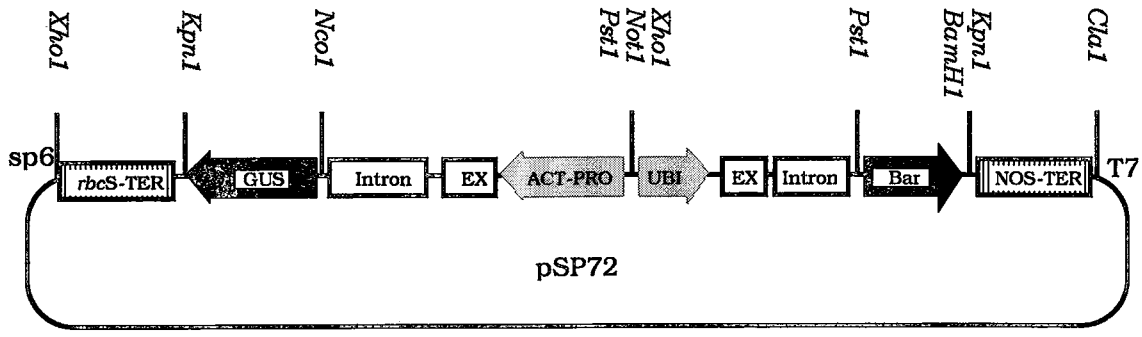

Fig. 1. Schematic structure and the restriction sites of the plasmid pDM803 (8.95Kb).
GUS: *E. coli* β-glucuronidase *uidA* gene, EX: enhancer, ACT-PRO: rice actin 1 promotor, UBI: maize ubiquitin 1 promoter, Bar: *Streptomyces hygroscopicus* phosphinothricin acetyltransferase gene, NOS-TER: nopaline synthase (*nos*) gene terminator, *rbcS*-TER: rice rubisco gene terminator. All of gene sequence inserted the region between the restriction sites *Cla1* and *Xho1* of clone plasmid pSP72, which is not drawn to scale.

(Christensen et al. 1992) with *Agrobacterium tumifaciens* nopaline synthase (*nos*) terminator (Bevan et al. 1983) and the *E.coli uidA* (β-glucuronidase) gene (Jefferson et al. 1987) under control of the rice *Act1* promoter (McElroy et al. 1991) with 3' transcript termination region of rice rubisco gene terminater (*rbcS*-TER). One enhancer was, respectively, inserted at the upstream of β-glucuronidase and *bar* to enhance their expression.

Particle bombardment: For each bombardment, 40 to 50 scutella were placed in the center of a 9 cm diameter Petri dish containing MS-based induction medium with 1 mg/l 2,4-D. Explants were cultured in dark at 25°C for 10 day prior to bombardment. Plasmid DNA was precipitated onto 1 mm diameter gold particles immediately prior to bombardment as detailed in Sanford et al. (1987). Bombardments were carried out using a PDS1000/He particle gun (Bio-Rad) at an acceleration pressure of 1,100 or 1,350 psi and a distance of 9cm from the stopping plate. After bombardment, explants were spread over the surface of the original medium and cultured at 25°C in darkness for 3 weeks to induce embryogenesis.

Histochemical GUS analysis: A histochemical GUS assay was conducted according to Jefferson et al. (1987). Immature embryos and calli were immersed in a 0.1 M phosphate buffer solution, pH 7.2, containing 1 mg/ml 5-bromo-4-chloro-3-indolyl glucuronide (X-gluc) at 37°C for 24 h in dark, subsequently washed in 70% alcohol and observed under microscope.

Statistical analysis: A completely randomized design was used to estimate the culture responses of different explants. The frequencies of induced-callus were investigated when explants had been cultured on the callus induction medium for four weeks. The effects of genotypes on culture responses were determined by variance analysis and shortest significant ranges tests (Rong 1993).

Table 2. Comparison of different explants for callus induction

Genotypes	Embryos	Status	No. of embryos	Callus induction frequency (%)
Chuannong 16	Mature	+[†]	114	84.2
	Immature	+++	315	91.8
98-1266	Mature	+	134	80.5
	Immature	+++	846	93.9

[†] + represents a few embryonic calli, +++ represents many embryonic calli.

Results

Effects of different explants on callus induction: The frequencies of induced-callus were investigated when explants had been cultured on the callus induction medium for four weeks (Table 2). Only a few calli were induced from immature inflorescences, while most of immature inflorescences directly initiated the roots. Thus, the callus induction frequency of immature inflorescence had not calculated in this study. In two genotypes, Chuannong 16 and 98-1266, the average callus induction frequency of immature embryos were 91.8% and 93.9%, respectively, and higher than that of mature embryos. The status of callus derived from different explants inoculated on medium MS_1 was evaluated according to the method of Ye et al. (2001). Many embryonic calli (+++ status) were induced from immature embryo derived-callus, which was yellowish and more compact. It indicated that immature embryos were more suitable for callus induction than those of immature inflorescences and mature embryos. The hydrocolloid-like calli (+ status) with ivory-white were easily turned to brown and initiated roots. Based on these results, the immature embryos were selected as explants for further studies.

Effects of genotype and medium on culture responses of immature embryos: Using 8 genotypes and 6 media, the effects of genotypes and media on callus induction frequencies of immature embryos were investigated (Table 3). Significant differences of callus induction frequencies of immature embryos were detected. Among these genotypes, Chuannong 16 had the highest callus induction frequency, while Chuanmai 32 had the lowest callus induction frequency. The average callus induction frequencies of Chuannong 16 and Chuanmai 32 were 92.9% and 81.8%, respectively. There was no significant variation among the callus induction frequencies of 5 genotypes (Chuannong 16, Chuanyu 16, 80-8, Yiyuan 2 and 98-1266), while significant difference was detected between these genotypes and the other genotypes.

The significant effects of medium on callus induction were observed. Among 6 media, the frequencies of callus induction on MS_2 and $N6_0$ were higher than those of the others. The average frequencies of callus induction on MS_2 and $N6_0$ were 94.0% and 93.1%, respectively. The lowest frequency (83.6%) of callus induction was observed on $N6_1$. When MS_2 and $N6_0$ were selected for callus induction media, it was observed that the frequency of initiated

Table 3. Callus induction frequencies of immature embryos form different genotypes on different media

Genotypes	Media						Average
	$N6_0$	$N6_1$	MS_0	MS_1	MS_2	MS_3	
Chuannong 16	93.2	88.4	93.6	92.5	99.3	90.5	92.9a†
98-1266	96.1	89.8	83.3	84.0	91.1	83.4	88.0a
Y1496	94.6	51.0	89.7	86.3	92.3	88.8	83.8b
Yiyuan 2	97.1	91.1	89.6	86.8	94.0	91.1	91.6a
Chuanyu 16	93.8	90.8	91.9	89.4	98.8	91.8	92.7a
Chuanmai 32	79.8	86.3	77.2	66.5	97.0	83.7	81.8b
Chuanyu 12	93.4	73.2	82.0	84.2	88.4	82.5	83.9b
80-8	90.7	98.0	89.9	90.1	96.9	85.3	91.8a
Average	93.1a	83.6b	87.1b	85.0b	94.0a	87.1b	

† Means with the same letters are not significantly different from each other according to the SSR test (at the 5% level)

Table 4. Frequency (%) of green shoot differentiation from calli on MS_{10} and $MS_{10\text{-}1}$

Media	Genotypes								Average
	Chuanyu 12	Chuannong 16	Y1496	98-1266	Chuanyu 16	Schuanmai 32	Yiyuan 2	80-8	
MS_{10}	83.3	77.8	80.0	69.2	40.0	50.0	75.0	42.1	64.9
$MS_{10\text{-}1}$	66.7	33.3	28.6	40.0	16.7	38.5	87.5	41.7	40.2

shoot directly from explants on $N6_0$ (60.2%) was higher than that of MS_2. It indicated that MS_2 was superior to $N6_0$ for callus induction. Thus, we chose MS_2 as callus induction medium in the following procedures. The plantlet regeneration competence of callus derived from different genotypes: In order to increase the

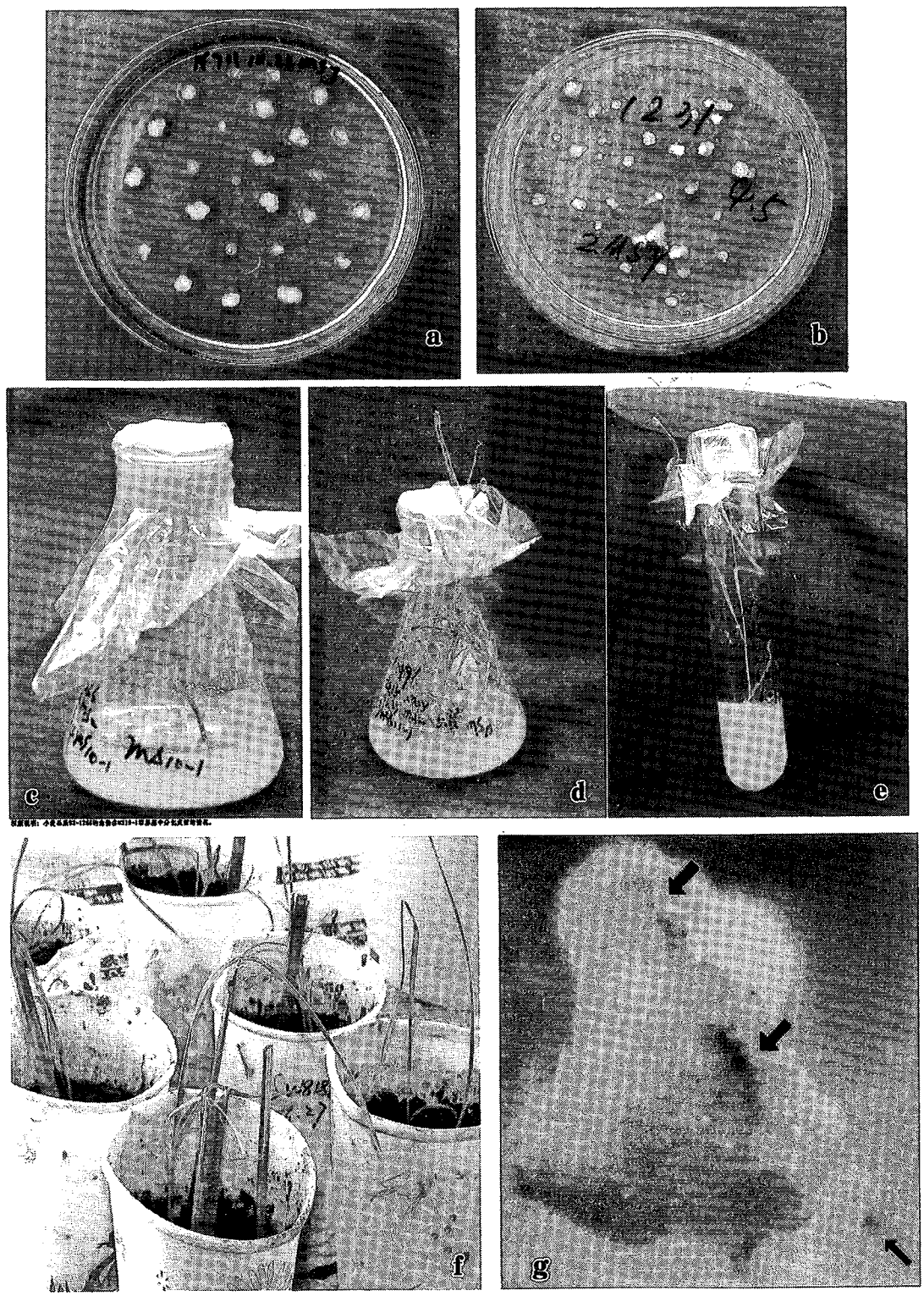

Fig. 2. Calli and green shoots regenerated.
a: The status of callus induced from mature embryo,1231 is the breeding number of Chuannong 16, b: The status of callus induced from immature embryo, c: The green shoot differentiate on callus, d: Multiple shoot, e: Plantlet regeneration, f: Plant transplantation, g: Transient expression of Gus after 7 days of bombed callus, black arrows indicated the blue spots of GUS activity.

number of in vitro plantlets and to investigate the green shoot differentiation competence of callus derived from medium MS_2, two regeneration strategies have been applied in this study: (1) Medium MS_{10} containing 2 kinds of auxins, 6-BA (2 mg/l) and IBA (0.5 mg/l), which could promote the shoot-like embryoid appearance, was used to induce green shoot at first step. When the shoot grow to 1–2 cm, the shoot with callus were transferred to MS_{11} or $MS_{11\text{-}1}$ for root initiation, then regenerated the young plantlets; (2) Medium $MS_{10\text{-}1}$ with 2 mg/l NAA and 0.5 mg/l KT was used for the induction of shoot-like and root-like embryoid at the same time. When the calli were transferred to MS_{10} or $MS_{10\text{-}1}$ for two weeks, the green shoots on calli of 8 genotypes were investigated (Table 4). On MS_{10}, Chuanyu 12 had the highest frequency of green shoot differentiation (83.3%). On $MS_{10\text{-}1}$, Yiyuan 2 had the highest frequency of green shoots differentiation (87.5%). Comparison of the differentiation frequency for each genotype on two media, it indicated that MS_{10} was superior to $MS_{10\text{-}1}$ for shoot differentiation. Only one genotype, Yiyuan 2, induced more green shoots on $MS_{10\text{-}1}$ (87.5%) than MS_{10} (75.0%)

Transient expression of GUS in particle bombarded calli: Some calli derived from immature embryos of Chuannong 16 were randomly chosen for transformation via particle bombardment. After one week of bombardment, the β-glucuronidase (GUS) activity was detected and observed as blue spots through histochemical assays. The control explants were bombarded without plasmid DNA and blue spots were not observed under the microscope. All spots were dark blue in bombarded immature embryo (Fig. 2 g). The mean frequency of blue spots was 5.6%.

Discussion

Successful wheat transformation is strongly dependent on a number of factors such as the genotype, medium composition, explants resources and transformation protocols. In this study, immature embryo, mature embryo and immature inflorescence of Chinese elite spring wheat genotypes were chosen as transgenic explant resources via in vitro culture. Only a few calli could be induced from immature inflorescences and the callus induction frequency was very low, while higher callus induction frequencies were obtained from immature inflorescences by Dudits et al. (1975) and Liu et al. (2001). We also found that the frequency of callus induction from immature embryos was higher than that of mature embryos and immature inflorescences. It suggested that immature embryos as transgenic explants were superior to mature embryos and immature inflorescences. The similar results had been reported by Altpeter et al. (1996), Chen et al. (1998) and Liu et al. (2002).

The frequency of stable wheat genetic transformations via particle bombardment is also strongly dependent on genotypes (Iser et al. 1999). The application of this technology had been made in genetic transformation of some European and Japanese elite wheat varieties with acceptable agronomic characters (Takumi and Shimada 1997; Iser et al. 1999). Our experiment found that Chuannong 16 was a good genotype for genetic transformation, and MS_2 medium was superior to other media for the callus induction. Chuannong 16 is a new breakthrough cultivar with high steady yield, large number of spikes per hectare and resistant to disease. It was registered by National Variety Identify Committee of China, and was released by our research group in 2002 (Zheng et al. 2002). Thus, this elite cultivar could be chosen as an ideal transformation genotype for further Chinese spring wheat genetic transformation. Several reports have focused on the examinations of medium parameters (Perl et al. 1992; Rasco-Gaunt et al. 1999; Qin and He 2001). General MS is main salt formulation used for cereal species from the Triticeae. Wu et al. (2003a, b) reported that modified N6 medium is the suitable medium for the wheat commercial cultivars, landraces and breeding lines in Sichuan, China. However, our experiment indicated that the MS with double macro salt was superior to other media and had higher calli induction frequency than that of general MS and N6 media.

The transient expression of GUS indicated there was a strong GUS positive response and this optimal tissue culture system was available for Chinese spring wheat genetic transformation via particle bombardment. Now, this tissue culture system has been used in the genetic transformation of high-molecular-weight (HMW) glutenin subunits gene derived from the wild relative genus of wheat by our research group (Yan et al. 2002; Liu et al. 2003) in order to improve the wheat quality of southwest China.

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References

Altpeter F, Vasil V, Srivastava V, Stöger E and Vasil IK (1996) Accelerated production of transgenic wheat (*Triticum aestivum* L.) plants. Plant Cell Reporter16: 12-17.

Barro F, Rooke L, Békés F, Gras P, Tatham AS, Fido R, Lazzeri PA, Shewry PR and Barcelo P (1997) Transformation of wheat HMW subunit genes results in improved functional properties. Nature Biotechnol 15: 1295-1299.

Bedö Z, Vida Gy, Láng L and Karsai I (1998) Breeding for bread making quality using old Hungarian wheat varieties. Euphytica 100: 179-182.

Bevan M, Barnes WM and Chilton M (1983) Structure and transcription of the nopaline synthase gene region of T-DNA. Nucleic Acids Res 11: 369–385.

Blechl AE and Anderson OD (1996) Expression of a novel highmolecular-weight glutenin subunit in transgenic wheat. Nature Biotechnol 14: 875-879.

Chen LH, Wang XW, Zhang XD, Hu DF and Liu GT (1998) Transformation of different acceptors in wheat following particle bombardment. Acta Agric Boreali- Sinica 13(1): 1-5.

Christensen AH, Sharrock RA and Quail PH (1992) Maize polyubiquitin genes: genes, structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer toprotoplasts by electroporation. Plant Molec Biol 18: 675–689.

Dudits D, Német G and Haydu Z (1975) Study of callus growth and organ formation in wheat (*Triticum aestivum*) tissue cultures. Canad J Bot 53: 957-963.

FAO (2003) Statistical Data Bases: Agriculture. http:// apps.fao.org

Hess J and Carman J(1998) Competence of immature wheat embryo: genotype, donor plant environment and endogenous hormone levels. Crop Sci 38: 249-253.

Iser M, Fetting S, Scheyhing F, Viertel K and Hess D (1999) Genotype-dependent stable genetic transformation in German spring wheat varieties selected for high regeneration potential. J Plant Physiol 154: 509-516.

Jefferson RA, Kavanagh TA and Bevan MW (1987) GUS fusion: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 6: 3901-3907.

Lazzeri PA, Barcelo P, Barro F, Rooke L, Cannell ME, Rasco-Gaunt S, Tatham A, Fido R and Shewry PR (1997) Biotechnology of cereals: genetic manipulation techniques and their use for the improvement of quality, resistance and input use efficiency traits. Aspects of Appl Biol 50: 1–8.

Leckband G and Löerz H (1998) Transformation and expression of a stilbene synthase gene of *Vitis vinifera* L. in barley and wheat for increased fungal resistance. Theor Appl Genet 96: 1004-1012.

Liu LX, Zheng QC, Zhao L, Zhang YF, Wang J, Zhao SR and Chen WH (2001) Study on receptor system for wheat genetic transformation without genotype dependence. Acta Agric Nuclearae Sinica 15(5): 308-310.

Liu WH, Li WX, Hu SL, Xu XL and Li JL (2002) Study on influncing factors of tissue culture and biolistic bombardment in wheat. Acta Bot Boreal-Occident Sinica 22(3): 602-610.

Liu Z, Z Yan, Y Wang, K Liu, Y Zheng and D Wang (2003) Analysis of HMW glutenin subunits and their coding sequences in two diploid *Aegilops* species. Theor Appl Genet 106: 1368-1378.

McElroy D, Blowers AD, Jenes B and Wu R (1991) Construction of expression vectors based on the rice actin 1 (*Act1*) 5' region for use in monocot transformation. Molec General Genet 231: 150-160.

McIntosh RA(1998) Breeding for resistance to biotic stresses. Euphytica 100: 19-34.

Perl A, Kless H, Blumenthal A, Galili G and Galun E (1992) Improvement of plant regeneration and GUS expression in scutellar wheat calli by optimizations of culture conditions and DNA-microprojectile delivery procedures. Molec General Genet 235: 279-284.

Qin JB and He GY(2001) Preliminary study on *in vitro* culture of different wheat genotypes and their explants. Jour Huazhong Agric Univ 20(6): 522-527.

Rasco-Gaunt S, Riley A, Lazzeri P, Baroelo P and De Block M (1999) A facile method for screening for phosphinothricin (PPT)-resistant transgenic wheats. Molec Breed 5: 255-262.

Rasco-Gaunt S, Riley A, Cannell M, Barcelo P, Lazzeri PA (2001) Procedures allowing the transformation of a range of European elite wheat (*Triticum aestivum* L.) varieties via particle bombardment. J Exp Bot 52: 865-874.

Rong TZ (1993) Statistical analysis and field experiment. Sichuan science & technology publishing. Chengdu China pp: 95-155.

Sanford JC, Klein TM, Wolf ED and Allen N (1987) Delivery of substances into cells and tissues using a particle bombardment process. Partic Sci Technol 5: 27–37.

Takumi S and Shimada T (1997) Variation in transformation frequencies among six common wheat cultivars through particle bombardment of scutellar tissues. Genes Genet Syst 72: 63-69.

Thompson CJ, Novva NR, Tizard R, Crameri R, Davies JE, Lauwereys M and Botterman J (1987) Characterization of the herbicide-resistance gene *bar* from *Streptomyces hygroscopicus*. EMBO Jour 6: 2519–2523.

Vasil IK(1998) Biotechnology and food security for 21st century: a real-world perspective. Nature Biotechnol 16: 399-400.

Vasil V, Castillo AM, Fromm ME and Vasil IK (1992) Herbicide resistant fertile transgenic wheat plants obtained by microprojectile bombardment of regenerable embryogenic callus. Biotechnol 10: 667-674.

Wu BH, Zheng YL, Liu DC and Zhou YH (2003a) Trait correlation of immature embryo culture in bread wheat. Plant Breed 122(1): 47-51.

Wu BH, Zheng YL, Liu DC, Zhou YH and Yan ZH (2003b) Unisexual pistillate flower regeneration in immature embryo culture of wheat . Acta Botanica Sinica 45(4) : 452-459.

Yan ZH, Wan YF, Liu KF, Zheng YL and Wang DW(2002) Identification of a novel HMW glutenin subunit and comparison of its amino acid sequence with those homologous subunits. Chinese Sci Bull 47 (3): 220-225.

Ye XG, Shirley S, Xu HJ, Du LP and Tom C (2001) Regular production of transgenic wheat mediated with *Agrobacterium tumefaciens*. Scientia Agricultura Sinica 34 (5) : 465-468.

Zheng YL, Lan XJ, Wei YM, Zhou YH, Liu DC, Yang JL and Yan J (2002) Analysis on agronomic characters of new wheat variety Chuannong 16. Jour Sichuan Agric Univ 9(3): 194-196.

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The effect of polyethylene glycol on seed germination of wheat (*Triticum aestivum* L.) genotypes/lines

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Water is generally considered as one of the limiting factors which affects the physiological and biochemical processes affecting crop productivity (Osborne et al. 2002). Water stress impairs seed germination, retards plant development and reduces crop yield (Bradford 1994). It also affects several morphological, biochemical alterations in plants ultimately leading to a massive loss in yield. If agriculture is to feed the world's burgeoning population, yields of water-limited crops must be improved substantially.

Seed germination is considered to be the most critical stage especially under stress conditions. The first requirement of seed germination is water for hydrolysis of reserves, as a medium of translocation and hydration of enzymes, for operational conformation of cell membranes, organelles and finally to provide the driving force for cell expansion reduced by germination. During germination, biochemical changes take place, which provides the basic framework for subsequent growth and development. The initial metabolic changes that occur immediately after the imbibition of water are the increase in the hydrolytic enzymes such as alpha-amylase and protease. Alpha-amylase is an important starch degrading enzyme in the endosperm of cereal grains. The reaction products provide substrate and an energy source for the embryo during germination (Ahmed and Bolton 1988). Hydrolysis and solubilization of starch during seed germination is crucial because of the activity of starch hydrolyzing enzyme alpha-amylase. The synthesis of this enzyme during germination is regulated by gibberellic acid (Chrispeels and Varner 1976) and hence starch hydrolysis. Inhibition of seed germination of crop plants is also due to disturbance in the activities of peroxidase, alpha-amylase and acid-phosphatase (Murata et al. 1968). It has been suggested the phenolics as the cause of inhibition of metabolic process during germination (Rice 1984). Generally, the phenolic compounds caused interference with IAA metabolism, mitochondrial function, synthesis of protein and ion uptake and transport (Einhelling 1986).

Drought stress can affect seed germination through osmotic effects (Welbaum et al. 1990) or by ionic toxicity (Bliss et al. 1986; Huang and Reddman 1995). Physiological studies to distinguish between the two effects are limited (Bliss et al. 1986), but evidence suggests that low water potential of the germination medium is a major limiting factor (Bradford 1994). In the present study, the drought stress delayed germination in all genotypes/lines to varying degrees.

One approach to reduce the deleterious effects of water stress or drought on crop production is the development of drought tolerant cultivars (Begg and Turner 1976). Varietal differences in drought resistance have been reported in wheat (Steiner et al. 1990), which can be further exploited by appropriate breeding programs to develop new varieties. Since polyethylene glycol of high molecular weight (PEG-6000) has been found to be most satisfactory in creating water deficit (Janes 1974), therefore it is used in different concentrations, 0.0, 0.25, 0.5, 0.75 and 1.0 MPa to create artificial stress.

Preliminary laboratory experiment was conducted to screen out 170 wheat genotypes/lines collected from different organizations of the country and those developed at NIA, Tandojam.

Good healthy wheat seeds were manually selected and surface sterilized with 5% sodium hypochlorite

Table 1. Germination (%) under stress condition

Varieties/Lines	0.0 MPa		0.25 MPa		0.5 MPa		0.75 MPa		1.0 MPa	
WL-711	100.0	a	93.3	abcdef	87.5	cdefgh	62.1	no	43.5	ef
Zardana	99.4	ab	97.3	abcd	86.3	efghij	77.1	bcdefg	34.6	ghijkl
V-7003	98.8	ab	94.4	abcde	76.5	mnopqr	60.9	op	51.3	d
V-7004	99.4	ab	98.3	abc	87.5	cdefgh	77.7	bcdef	28.1	klmnopqr
V-7012	99.2	ab	95.2	abcde	72.3	opqrs	65.6	klmno	00.0	[
V-7015	98.8	ab	96.7	abcd	76.9	lmnopqr	61.3	O	00.0	[
V-8001	99.6	ab	94.0	abcde	84.0	efghijkl	75.0	cdefghi	00.0	[
V-8003	98.3	ab	95.0	abcde	81.9	ghijklm	74.4	cdefghi	6.9	xyz
V-8004	100.0	ab†	99.2	ab	94.0	abc	63.5	mno	54.4	d
G.P-2	99.6	ab	99.0	ab	87.9	bcdefgh	60.2	op	11.9	wxy
G.P-7	99.6	ab	92.3	bcdefgh	81.3	ghijklm	75.0	cdefghi	11.9	wxy
A.G.A	99.4	ab	96.3	abcd	94.8	ab	77.1	bcdefg	0.0	[
Bucs	99.4	ab	96.5	abcd	90.6	abcde	80.2	abcd	17.1	uvw
C-228	100.0	a	98.3	abc	93.8	abcd	72.7	efghij	40.4	efg
C-591	100.0	a	98.8	abc	94.8	ab	82.7	ab	100.0	a
Chakwal-86	98.5	ab	97.3	abcd	90.0	abcdef	82.3	ab	6.9	xyz
C.M-24/87	99.6	ab	96.9	abcd	86.9	cdefghi	77.5	bcdef	73.1	b
D.S-11	100.0	a	99.2	ab	78.1	klmnop	72.3	efghijk	27.5	lmnopqr
H-68	99.6	ab	98.0	abc	89.8	abcdef	79.4	abcde	0.0	[
P-15800	99.6	ab	97.1	abcd	82.1	ghijklm	75.9	bcdefgh	3.1	z [
P.K.V-1600	99.8	a	93.5	abcdef	86.7	defghi	73.1	defghij	5.6	yz [
Q.M-4531	99.2	ab	96.9	abcd	88.5	bcdefg	78.5	abcde	72.3	b
Q.M-4934	98.5	ab	95.4	abcde	90.4	abcde	77.1	bcdefg	70.8	bc
R.G-24	100.0	a	100.0	a	96.3	a	84.8	a	74.8	b
SARC-I	98.3	ab	97.5	abcd	93.8	abcd	80.8	abc	69.4	bc
V-8319	100.0	a	96.9	abcd	84.4	efghijk	77.7	bcdef	65.0	c
M-172	99.4	ab	92.7	abcdefg	82.7	fghijklm	70.6	fghijkl	54.4	d
H.T-37	98.8	ab	77.5	mn	80.0	ijklmn	76.3	bcdefgh	35.6	ghij
H.T-29	93.8	ab	78.6	lmn	77.5	klmnopq	69.4	hijklm	22.5	qrstu
E.S.W-9525	98.8	ab	81.9	jklm	73.3	nopqrs	64.4	lmno	37.7	fghi
Z.A-77	99.8	a	93.5	abcdef	84.0	efghijkl	68.3	ijklmn	25.8	nopqrs

† Means followed by same letters do not differ significantly at the 5% level according to Duncan multiple range test.

(NaOCl) solution for 10 minutes, washed with distilled water several times, and briefly blotted into fine quality filter paper. Seeds were germinated in covered sterilized Petri dishes containing germination paper moistened with 10ml of different concentrations of PEG-600 separately. There were 20 seeds of each wheat genotype placed in Petri dish with three replications covered with black muslim cloth and then kept in an incubator in randomized block design for 8 days at 25/20°C day/night temperature. Seeds were considered germinated when the emergent radicle reached 2mm in length. Germination percentage was recorded after 192 hours (8 days) of growth.

Germination of 31 wheat genotypes/lines, which were screened out from 170 genotypes/ lines has been shown in Table 1. In all wheat genotypes/lines, the germination percentage decreased with increasing osmotic stress. It was observed that 31 genotypes/ lines showed more than 60 percent germination at 0.5 and 0.75 MPa, however C-591, CM-24/87, QM-4531, QM-4934, RG-24, SARC-I, V-8319, M-172 performed well even at 1.0 MPa demonstrating high genetic potential for drought resistance during germination.

According to Wiggns and Gardner (1959), water stress can affect germination by delaying initiation. Cianiprorova and Luxova (1976) noted cessation of root growth in maize plant when exposed for 24–48 hours to PEG solution as a result of the inhibition of cell elongation and division. The most tolerant variety

was C-591 having 100 percent germination even at the highest water stress level (1.0 MPa), when compared with control. Similarly, the most sensitive varieties were found to be V-8001, AGA, H-68 at highest water stress level (1.0 MPa). It was also observed that degree of reduction increased proportionally with increasing concentration of PEG-6000 solution in the growth medium.

References

Ahmed S and Bolton FE (1988) Physiological differences in germinating wheat seeds due to seed source. Pak J Bot 20(2): 227-231.

Begg JE and Turner NC (1976) Crop water deficit. Adv Agron 28: 161-217.

Bli RD, Platt-Aloia KA and Thomson WW (1986) Osmotic sensitivity in relation to salt sensitivity in germinating barley seeds. Plant Cell Environ 9: 721-725.

Bradford KJ (1994) Water stress and the water relations of seed development: A critical Review. Crop Sci, 34: 1-11.

Chrispeels MY and Varner JE (1976) Gibberellic acid enhanced synthesis and release of alpha-amylase and ribonuclase by isolated barley aleuron layers. Plant Physiol 42: 398-406.

Cianiprorova M and Luxova (1976) The effect of polyethylene glycol induced water stress on the maize root apex. Biologia Plant 18: 173-178.

Einhelling FA (1986) Mechanism and modes of action of allelochemicals In: Putnam AR and Tang CS (ed) The Sci Allelopathy, John Wiley and Sons, New York: 171-188.

Huang J and Reddman RE (1995) Salt tolerance of *Hordeum* and *Brassica* species during germination and early seedling growth. Can J Plant Sci 75: 815-819.

Janes BE (1974) The effect of molecular size, concentration in nutrient solution and exposure time on the amount distribution of polyethylene glycol in Pepper plant. Plant Physiol 54: 226-240.

Murata T, Akazawa T and Fukuchi S (1968) Enzymic breakdown of starch in germinating rice seeds. An analytical study. Plant Physiol 43: 1899-1905.

Osborne SL, SchepersV, Francis DD and Schlemmer MR (2002) Use of spectral radiance to estimate in-season biomass and grain yield in nitrogen and water-stressed crop. Crop Sci 42: 165-171.

Rice EL (1984) Allelopathy. 2nd ed. Academic Press. Orlando Florida, USA.

Steiner JJ, Hutmacher RB, Mantal AD, Ayars JE and Vail SS (1990) Response of seed carrot to various water regimes. II. Reproductive development, seed yield and seed quality. J Am Sco Hort Sci 115: 722-727.

Welbaum GE, Tissaoui T and Bradford KJ (1990) Water relations of seed development and germination in muskmelon (*Cucumis melo* L.). III. Sensitivity of germination to water potential and abscisic acid during development. Plant Physiol 92: 1029-1037.

Wiggns SC and Gardner FP (1959) Effectiveness of various solutions for simulating drought conditions as measured by germination and seedling growth. Agron. J : 315-318.

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Research information

Mapping of *TaHd1-1*, a wheat homologue of a rice gene that controls heading date (*Hd1*)

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The *Hd1* gene is a major photoperiod sensitivity gene in rice (Yamamoto et al. 1998; Yano et al. 2000). An ortholog of *Hd1* in wheat, designated as *TaHd1*, has been isolated and has been functionally analyzed by Nemoto et al. (2003). They found that there are three homoeologous *TaHd1* genes (*TaHd1-1*, *TaHd1-2*, and *TaHd1-3*) corresponding to the three homoeologous genomes (A, B and D) in wheat. By using nullisomic-tetrasomic (NT) and ditelosomic (DT) lines of a common wheat cultivar, Chinese Spring, the three homoeologous *TaHd1* genes were assigned to the group-6 chromosomes. An expression analysis indicated that, of the three homoeologous genes, only *TaHd1-1* and *TaHd1-3* were expressed in the seedling stage of Chinese Spring. However, no photoperiod response gene has been assigned so far to the group-6 chromosomes. When *TaHd1-1* was transferred into a rice line deficient in *Hd1* function, the transgenic plants complemented the function of *Hd1*; namely, *TaHd1-1* promoted heading under the short-day-length condition and suppressed heading under the long-day-length condition. It is necessary to examine the genetic effects of *TaHd1-1* under different photoperiod conditions and in different wheat genetic backgrounds. Toward this goal, we developed a PCR-based DNA marker for the direct targeting of *TaHd1-1* and mapped the *TaHd1-1* gene in a segregating wheat mapping population in the present study.

A common wheat cultivar Gamenya from Australia was used to identify the sequence polymorphism of the *TaHd1-1* gene in wheat. A doubled-haploid (DH) population derived from an F_1 cross between two common wheat cultivars Sumai #3 and Gamenya, developed by the wheat x maize system (Ban and Suenaga 2000), was used to map *TaHd1-1* with the developed DNA marker as well as the SSR, AFLP, and RFLP markers. To verify the variation in different wheat cultivars and to evaluate the potential of the application of the developed DNA marker in different wheat genetic backgrounds, the marker was tested for 14 common wheat cultivars derived from different countries.

The *TaHd1* genes were amplified with a primer pair 5'-AGCTCACTTAGGATACAAGATGC-3' and 5'-CCTCCATTGATGAGAAAGATAT-3' under the same PCR conditions described by Nemoto et al. (2003). When PCR products were separated on a 1.5% agarose gel, a DNA fragment corresponding to the *TaHd1-1* gene was excised from the gel. DNA that was purified from the gel was used as a template for a second PCR with the same primer pair. The second-round PCR products were cloned into vectors with the pGEM-T Easy kit (Promega, Madison, WI, USA). Cloned fragments were subjected to sequencing with the CEQ™ sequencing kit (Beckman Coulter, Fullerton, CA, USA) on the CEQ™ 2000 DNA Analysis System following the manufacturer's instructions. The obtained DNA sequences were aligned with the sequence of *TaHd1-1* in Chinese Spring (AB094487) using the program GENETYX-MAC (Version 8.0, SOFTWARE DEVELOPMENT CO., LTD.).

A total of six sequence mutations were detected between Gamenya and Chinese Spring. Four of the mutations had single-base substitutions, one mutation had an insertion/deletion, and one mutation had different numbers of single-base repeats, 17 'G' in

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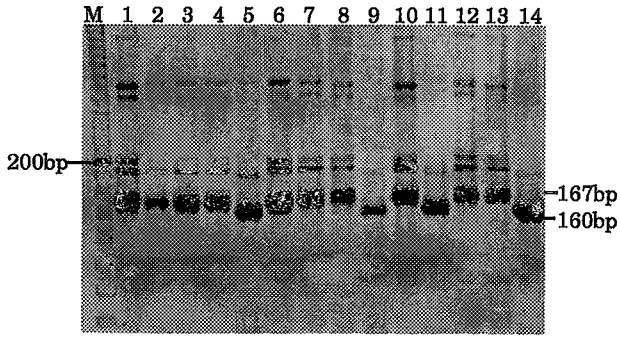

Fig. 1. PCR band patterns of the *TaHd1-1* gene in 14 common wheat cultivars from different countries.
Lane 1: Chinese Spring, 2: Hokushin, 3: 14006, 4: Kitakei 1657, 5: Norin 59, 6: Soujukuakage, 7: Asakazekomugi, 8: Catbird, 9: Chili, 10: Emblem, 11: MN2535, 12: Nobeokabouzukomugi, 13: Sumai #3, 14: Gamenya, M: 20-bp DNA ladder. PCR products were separated on a 15% acrylamide gel.

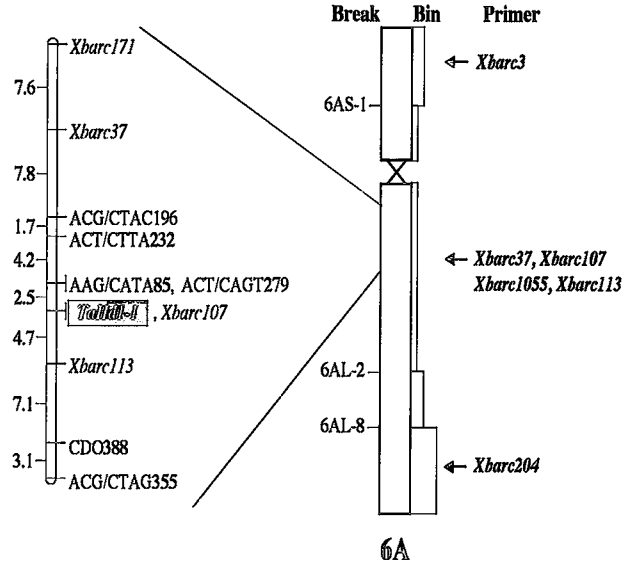

Fig. 2. Map position of the *TaHd1-1* gene (highlighted) determined with a DH mapping population derived from a cross between Sumai #3 and Gamenya.

Chinese Spring and 10 'G' in Gamenya. We designed a primer pair (5'-TAGGCAGCTTGTTCCTCTGC-3' and 5'-TCAAAGTCCATTGACTACCATCC –3') flanking the latest mutation and conducted PCR with the primers in 14 common wheat cultivars including Chinese Spring and Gamenya. Four cultivars showed the Gamenya genotype and 10 cultivars had the Chinese Spring genotype (Fig. 1). Since this marker also gave a polymorphism between Gamenya and Sumai #3, we applied the DNA marker to a mapping analysis with the DH population derived from a cross between Sumai #3 and Gamenya. With the *Xbarc* series of SSR markers (Song et al. 2000; Shi et al. 2003) and an RFLP marker (Mingeot and Jacquemin 1999) as anchor markers, *TaHd1-1* was mapped to the chromosome 6AL and it co-segregated with *Xbarc107* (Fig. 2). This result confirmed the result of the aneuploid analysis by Nemoto et al. (2003).

Although Nemoto et al. (2003) found that transgenic rice with *TaHd1-1* complemented the deficiency of *Hd1*, we did not detect an association between *TaHd1-1*and heading date in the DH population derived from Sumai #3 and Gamenya that was grown under natural conditions in Tsukuba, Ibaraki, Japan, in 2002 and 2003 (unpublished data). The DNA marker for *TaHd1-1* developed in the present study, as well as the flanking SSR and RFLP markers, will be useful for studies on the genetic effect of *TaHd1-1* under different photoperiod conditions and in different wheat genetic backgrounds.

References

Ban T and Suenaga K (2000) Genetic analysis of resistance to Fusarium head blight caused by *Fusarium graminearum* in Chinese wheat cultivars Sumai #3 and the Japanese cultivar Saikai 165. Euphytica 113: 87-99.

Mingeot D and Jacquemin JM (1999) Mapping of RFLP probes characterized for their polymorphism on wheat. Theor Appl Genet 98: 1132-1137.

Nemoto Y, Kisaka M, Fuse T, Yano M and Ogihara Y (2003) Characterization and functional analysis of three wheat genes with homology to the CONSTANS flowering time gene in transgenic rice. Plant J 36: 82-93.

Shi JR, Song QJ, Singh S, Lewis J, Ward RW, Cregan P and Gill BS (2003) Genetic and physical maps of *Xbarc* SSR loci in wheat. 2003 National Fusarium Head Blight Forum Proceedings, Bloomington, MN, USA: 41-45.

Song QJ, Fichus EW and Cregan PB (2000) Construction of genomic libraries enriched with microsatellite sequences. 2000 National Fusarium Head Blight Forum Proceedings, Erlanger, KY, USA: 50-51.

Yamamoto T, Kuboki Y, Lin SY, Sasaki T and Yano M (1998) Fine mapping of quantitative trait loci *Hd-1*, *Hd-2* and *Hd-3*, controlling heading date of rice, as single Mendelian factors. Theor Appl Genet 97: 37-44.

Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L, Fuse T, Baba T, Yamamoto K, Umehara Y, Nagamura Y and Sasaki T (2000) *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANS*. Plant Cell 12: 2473-2483.

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Research information

New sources of leaf rust resistance in Indian local durum wheat

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Leaf rust (*Puccinia triticina* Eriks. = *P. recondita* f.sp. *tritici*) is the most important disease of wheat. Race 77 complex of leaf rust has knocked down almost all the present bread wheat varieties in the country, creating an alarming situation. Durum wheats showed high levels of resistance to the leaf rust race 77 pathotypes (Pandey and Rao 1984; Honrao and Rao 1996; Nayar et al. 1996; Sharma et al. 1996). Identification of appropriate pathotypes of leaf rust for evaluating resistance in seven Indian local durum wheats was carried out based on seedling tests of 33 currently evolved pathotypes as well as their field response during 1997–2002 seasons. Hence, it is important to identify new germplasm for breeding of durums possessing resistance to leaf rust. The present communication reports seven new genetic stocks of Indian local durums with leaf rust resistance; A090, Dasarkhed-1, Haura, Bansi-288-18, Bijaga red, Bansi 207-3 and Malvi local (Table 1).

These varieties showed high level of field resistance with mixture of races at Pune during 1997–2002 with artificial epiphytotic conditions (Table 2).

Seedling response to 33 important Indian leaf rust pathotypes were also evaluated along with suitable

Table 1. Characterstics of new germplasm of Indian local *durums* with leaf rust resistance

Genetic stock	Parentage/Source	Phenotypic traits
1 A090	Local variety from the germplasm collection at ARI, Pune	Medium tall (<100 cm), medium early flowering, long pubescent spike, yellow glume and yellow awns. Good quality with amber and bold grain.
2 Dasarkhed-1	Local variety from the germplasm collection at ARI, Pune	Medium tall (<100cm), long ears with non pubescent yellow glume and yellow awns. Flowering is medium early. Good quality, amber and bold grain.
3 Haura	Local variety from the germplasm collection at ARI Pune	Medium tall variety (<100 cm), long ear with pubescent spike, yellow glume, black awns. Flowering is medium early. Grain is very bold, good quality.
4 Bansi 288-18	Land race from Mundia area (Madhya Pradesh, India)	Tall in height (> 110 cm), long ears with pubescent spike with yellow glume and black awns. Medium quality wheat. Amber and bold grain.
5 Bijaga red	Mysore local/Gaza	Medium tall variety (<100 cm), medium long ear with non-pubescent spike, yellow glume and yellow awns. Red and bold grains, poor flour quality .
6 Bansi 207-3	Local variety from the germplasm collection at ARI Pune	Tall in height (> 110 cm), long ear with pubescent spike, yellow glume and black awns. Medium quality, amber and bold grain.
7 Malvi local	Land race from Malva area	Medium tall (< 100 cm), medium early flowering, long ear with yellow glume and black awns. Amber and bold grain.

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Table 2. Field response of durum wheat lines to stem rust and leaf rust during 1997-2002.

Durum wheat	Crop season									
	1997-98		1998-99		1999-2000		2000-2001		2001-2002	
	SR	LR	SR	LR	SR	LR	SR	LR	SR	LR
1 A090	10S	20MR	5S	TMR	TS	5MR	5S	F	SS	TR
2 Dasarkhed-1	5S	20MS	TS	5MR	F	10MR	20S	F	20S	5MR
3 Haura	20S	20MS	10S	5MR	5S	20MR	10S	TMR	20S	F
4 Bansi-288-18	30S	20MR	10S	F	10S	20MR	40S	TR	40S	5MR
5 Bijaga red	40S	F	20S	F	20S	F	30S	F	40S	F
6 Bansi 207-3	20S	20S	10S	5MR	TS	10MR	10S	TMR	30S	F
7 Malvi local	10S	F	5S	F	TS	TR	5S	F	5S	F
8 Gulab	60S	80S	40S	60S	80S	80S	80S	80S	80S	80S
9 Agra local	80S	80S	60S	60S	60S	80S	80S	80S	80S	80S

LR and SR = leaf rust and stem rust, respectively.
All local varieties showed resistance to leaf rust at adult plant stage (F to 20 MR-MS)

Table 3. Differences in seedling response of durum wheat lines to 33 test pathotypes of leaf rust.

Durum wheat lines		Spectrum of resistance to the 33 test pathotypes of leaf rust
1	A090	Resistant to all the pathotypes except 11,12-2, 12-4, 12A, 104-1, 104-2, 104-3, 106
2	Dasarkhed-1	Resistant to all the pathotypes except 12-2, 12A, 104-1, 104-2, 104-3, 106
3	Haura	Resistant to all the pathotypes except 11, 12, 12-2, 12-4, 12A, 104-1, 104-2, 104-3
4	Bansi 288-18	Resistant to all the pathotypes except 11, 12-2, 12A, 104-1, 104-2, 104-3
5	Bijaga red	Resistant to all the pathotypes except 77-6, 77-7, 104-2, 104-3, 104B
6	Bansi 207-3	Resistant to all the pathotypes except 11, 12, 12-2, 12A, 104-1, 104-2, 104-3
7	Malvi local	Resistant to all the pathotypes except 11, 12, 12-2, 12-4, 104-2, 104B, 106

checks at 20 ± 2°C temperature and 80% relative humidity controlled conditions by using standard procedure (Stakman et al. 1962) and further tested at DWR Regional Research Station Simla for confirmation. Differences in seedling response of durum wheat lines to 33 test pathotypes of leaf rust (Table 3).

Six of seven durum lines were highly resistant to all biotypes of race 77 and other pathotypes at seedling stage except Bijaga red, which was susceptible to 77-6 (121 R 55-1). All local durums showed susceptible reaction to weak pathotypes viz. 11(OR8), 12 (5R%) and 104 (17R23). However, the pathotype 77-5 (121R63-1) was avirulent to all the local durum lines. In contrast, the leaf rust pathotypes 11,12,104 and 106, presumed to be weak pathotypes due to their low levels of virulence to known leaf rust resistance genes, were more virulent to durums, compared to bread wheats.

As mentioned above, these local durums being promising for seedling as well as field resistance to leaf rust and possess desirable agronomic and quality characteristics, their use as breeding stocks for leaf rust resistance-cum-quality wheat breeding is suggested. The resistance from durums may be exploited to widen the genetic base of resistance in bread wheat.

References

Honrao BK and Rao VSP (1996) Sources of resistance to race 77 of leaf rust (*Puccinia recondite* f. sp. *tritici)* in durum wheat. I. Seedling resistance. Cereal Rusts and Powdery Mildews Bull, 24: 39-43.

Nayar SK, Prashar M, Kumar J, Bhardwaj SC and Verma LR (1996) Distribution pattern of *Puccinia recondite tritici* pathotypes in India during 1990-94. Ind J Agri Sci 66(10): 621-630.

Pandey HN and Rao MV (1984) Differential behaviour of *aestivum* and *durum* wheats to race 77 and 106 of leaf rust (*Puccinia recondite* Rob. ex. Desm.). Wheat Inf Serv 58: 34-35.

Sharma SC, Saini RG and Goel RK (1996) Diversity for new leaf rust resistance genes in some macaroni wheat accessions. Cereal Rusts and Powdery Mildew Bull 24: 35-38.

Stakman EC, Stewart DM and Loegening WQ (1962) Identification of physiologic races of *Puccinia graminis* var. *tritici.* USDA Agric Res Serv Bull E-617 (rev.): 1-53.

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Record

A joint meeting of the Japanese Wheat Genetics Symposium and Triticeae Molecular Biology Workshop, November 27-28, 2004

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A joint meeting of the 29th Japanese Wheat Genetics Symposium (JWGS) and 9th Triticeae Molecular Biology Workshop (TMBW) was held in Tottori, in November 27 and 28, 2004. The local organizing committee consisting of H. Tsujimoto (Chairman) and H. Tanaka were in charge of its organization and management. Ninety-nine researchers and students discussed on the following three topics. 1) Where is the interphase between genomics and production of wheat and barley? 2) New aspect of germplasm exploration, and 3) Insight from chromosome research. In addition, 18 posters and 18 preliminary reports were presented. All the attendees stayed together in Hotel Hope Star Tottori, enjoyed the meal, hot spring, and talking till all hours of the night. In the business session several agenda were discussed, and an agreement about the form and the site of the next meeting were made: From the next meeting JWGS and TMBW will be united and held in Kyoto Prefectural University in 2005 by Prof. Y. Ogihara. Followings are the abstracts of the symposia and poster presentation of this joint meeting.

Symposium I.
Where is the interphase between genomics and production of wheat and barley?

S-1-1 **T. Kaneko** (Bioresources Research and Development Laboratories, Sapporo Breweries LTD)
Barley material and brewering process

Beer is one of most popular alcohol beverage in the world. Malt which is produced from germinated barley (*Hordeum vulgare*) is an important raw material for beer brewing. It is not only a source of carbohydrate for yeast fermentation, which also plays functional rules of enzyme degradation and conversion of starch, protein and other seed contents to substances for brewing process. These complicate traits affect the character and quality of beer products. Therefore, continuous and various efforts have been paid for establish "malting" barley. The development of brewing technology causes new demand for the malting barley. The malting barley breeding applied with new technologies will continue as long as the brewing industry is developing.

S-1-2 **K. Hatta** (National Agricultural Research Organization, National Agricultural Research Center for Kyusyu and Okinawa region)
What would be demanded of anything or anyone in the connection of breeding objects and ideal genotypes in wheat?

At present, breeding of wheat is carried out at five National Agricultural Research Centers and at four Agricultural Experiment Stations in prefecture as a part of a national project in Japan. The breeding object of these programs is simple and clear: to produce the wheat varieties which have a high quality same as the imported wheat brand, ASW. Recently, we have got a powerful selection tool, the molecular technique, which is still being developed. It seemed that this tool brought a kind of dawn in the breeding world, but adoption of the molecular technique to practical wheat

breeding has been limited in Japan. One possible reason, especially in hexaploid wheat, would be the unclear relation between phenotypes we are looking for and ideal genotypes for them. To assist discussion of how we can take advantage of this technique practically, I introduce our classical selection methods for noodle qualities and some trials utilizing molecular assisted selection in our breeding programs.

S-1-3 **S. Ito** (Faculty of Agriculture, Tottori University)
World wheat at stake: - contemporary global grain supply demand competition -

World wheat production has been stagnated during the recent years. In 1997, the world production was the record at 610 million tons. However, since then, the record was never surpassed during the next 7 years, and a new record finally made in 2004 at 611 million tons, just barely above the 1997 level. Meanwhile, corn production has been steadily increasing relative to wheat. It recorded 600 million ton level in 1998, then it got leveled off for the next 4 years. However, it again started increasing thereafter setting another record at 664 million ton in 2004. Production is heavily related to change in prices. Lower prices are due to weak demand. During the first half of 1990's, wheat prices were cheaper, but consumption was stagnated. If this situation continues, wheat production would have to be downsized in the near future. Increases in feed consumption of wheat would be a solution.

S-1-4 **Y. Ogihara** (Laboratory of Genetic Engineering, Graduate School of Agriculture, Kyoto Prefectural University)
Toward functional genomics of polyploid wheat

In order to develop functional genomics of polyploid wheat, we have been systematically performing on large scale analysis of ESTs (transcriptome) in common wheat: (1) The cDNA libraries were constructed from the tissues during wheat life cycle and stress-treated. Up to present, 328,736 sequences were accumulated. These sequences were grouped into 32,881 gene clusters which are estimated to cover ca. 80 % of total wheat genes. Based on the constituent of contigs expressed in each of the cDNA libraries, gene expression profiles were inferred for contigs-tissues (Virtual Display). (2) Based on the 32,881 gene clusters, the Agilent oligomicroarrays with 22K and 11K wheat genes, which harbor independent clones with each other, were prepared. These oligomicroarrays are available from our laboratory. (3) The full length cDNA libraries were constructed from the two sources. One was constructed from the young spikelets. 24,056 ESTs from both ends of the cDNA clones were obtained. These ESTs were grouped into 1902 gene clusters. The other is a library of pooled RNAs extracted from 12 different tissues/stressed tissues. About 12,000 cDNA clones were one-pass sequenced from both ends and ca. 5000 independent clones were fully sequenced. Additionally, DNA sequencing of the three homoeologous chromosome regions in common wheat, where agronomically important genes are located, have been determined. Up to now, DNA sequence data of six genes (18 TAC clones) have been accumulated. Although most genes and/or ORFs were found among three homoeologous genomes, some ORFs existed in certain genomes. Furthermore, different expression patterns of some genes among genomes were observed. Repetitive sequences, especially retrotransposons around the genes were characteristic of each genome.

S-1-5 **H. Handa** (Department of Plant Biotechnology, National Institute of Agrobiological Sciences, Graduate School of Life and Environmental Sciences, Tsukuba University)
Comparative Genomics –from Rice to Wheat, from Wheat to Rice–

Rice and wheat belong in the same family, Poaceae. Therefore two plants share a substantial number of orthologous genes. However the pathways and the underlying networks may function in an alternative fashion. For instance, rice is a short-day plant and adapts to subtropic climate. On the other hand, wheat shows a heading under long-day condition and an adaptation to more temperate zone. By the end of this year, a "finished" rice genome sequence with 99.99% accuracy will come to us. In addition to the sequence, recent rice genome research has provided us much information. Wheat genome research has clearly benefited from rice genome research, both in the use of functional data and in research methodology. However, the advance in wheat genomics is also useful for the rice genomic research, because the progress of wheat genomics will provide more accurate insights into how different (or similar) the two plants are. For a broader understanding of genome function of rice and wheat, a comparative genomic study should be promoted, not only from rice to wheat, but also from wheat to rice.

S-1-6 **K. Sato** (Research Institute for Bioresources, Okayama University)

Anything different in research styles after analyzing barley genome?

Compared to model species for genome analysis like rice and *Arabidopsis*, genetics in Triticeae species are still in slow fashion. The impact of rice genome sequencing influenced the research style of barley especially genetic mapping and gene isolation. By the development of large-scale EST map, marker generation in barley is not a serious issue. The development of barley BAC libraries promotes us to clone genes. The combination of BAC library and EST mapping identifies gene-rich regions of the genome. After sequencing these regions, we will participate the research style of model species. Geneticists and breeders should aware of these facilities and try to avoid duplicated efforts. We must fine-tune the system of genome wide analysis on barley to be directed onto Triticeae genome. The hardwares are not available every research unit but the availability and awareness of these systems may be essential in the future research in Triticeae.

Symposium II.
New aspect of germplasm exploration

S-2-1 **S. Ohta** (Department of Bioscience, Fukui Prefectural University)

A report on the international cooperative field research project, Fukui Prefectural University Agro-ecological Exploration in Southwest Eurasia (FASWE)

International cooperative field research projects are proceeding in Turkey and Iran with the financial support by the JSPS (Grant-in-Aid No.15255012). The aim is to clarify interrelationships among environments, people, crops, their wild relatives and weeds in the agricultural system based on wheat and barley. In Turkey, cooperation is carrying out with Dr. Hakan Ozkan, Cukurova University. Southeastern Turkey was surveyed in 2003 and 2004. Allopatry of the two intraspecific taxa in *Aegilops neglecta* and dimorphism of seed dormancy in *Aegilops* species were revealed. In Iran, Dr. Javad Mozafari, National Plant Gene-Bank of Iran, cooperates with us. The Caspian Sea coast and the Zagros Mountains were surveyed in 2003 and 2004. Allopatry of the three intraspecific taxa in *Ae. tauschii* and geographical distribution of *Secale cereale* with brittle and non-brittle spikes were revealed. Intraspecific genetic diversity in crops, their wild relatives and weed species will be studied until March of 2007.

S-2-2 **K. Kato** (Faculty of Agriculture, Okayama University)

Field study on genetic diversity of wheat in East Asia

China is diversified in topographical features and climate, ranged from 150 m below sea level to 4000 m above sea level, from 5.8°C to 26.4°C in average annual temperature, and 4 mm to 6558 mm in average annual precipitation (Li et al. 1998). Reflecting such diversity, different types of wheat are cultivated in respective areas in China. To uncover genetic diversity among wheat and barley landraces in and around China, field study has been carried out in Tibet (1995, 1996, 1998), Shaanxi (1998, 1999) and Yunnan (2004), China, in Nepal and Bhutan (1999), and in India (2004). These field studies have been carried out as one of the activities of research projects entitled as "Genetic assay and study of crop germplasm in and around China" and "The development of sustainable biological production technologies harmonized with regional environmental conditions in East Asia", both of which were supported by a Grant-in-Aid of Ministry of Education, Science, Culture and Sports, Japan.

Symposium III.
Insight from chromosome research

S-3-1 **T. Wako** (Department of Biochemistry, National Institute of Agrobiological Sciences)

Histone code for chromosome structure and function

Control of gene expression is essential for improvement of crop production, such as expression of useful endogenes and/or transgenes, and repression of harmful endogenes and transposable elements. For this purpose, not only effective DNA code such as useful genes and regulatory elements but also histone code should be investigated. Histone code is defined certain combinations of post-translational modifications in nucleosomal histones. It affects chromosome and chromatin structure, and regulates

epigenetically various cellar functions such as gene transcription. To reveal histone code for chromosome structure and function, the dynamics of histone H4 acetylation at lysine residues in barley mitotic chromosome was analyzed by using three-dimensional immunofluorescent method. Pair-wise dynamics of histone acetylation were observed at nucleolar organizing regions and distal regions of chromosome in stage-specific manner. These pair-wise acetylations may regulate chromosome structure and function during mitosis as histone code.

S-3-2 **K. Nagaki** (Research Institute for Bioresources, Okayama University)
Exploration into the last frontiers of complex eukaryotic genomes

Centromeres are the last frontiers of higher eukaryotic genomes, consisting of highly repeated sequences that resist mapping, cloning and sequencing. Since it was believed as the centromeres are heterochromatic and do not contain any important active genes, the regions were left out from genome projects. We analyzed the regions in *Arabidopsis* and maize, and found that tandem repeats are functional. However, we could not know entire structure of the centromeres and size of the functional region because of the difficultness. The centromere of rice Chromosome 8 (Cen8) is a good example to know them. The Cen8 has an unusually low abundance of highly repetitive satellite DNA, which allowed its sequence to be determined. A ~750 kb region within the Cen8 binds rice CENH3, the centromere-specific H3 histone. The CENH3 binding region is contained within a larger heterochromatin. Surprisingly, we found fourteen predicted and four active genes in the CENH3 binding region.

S-3-3 **S. M. S. Tomar[1], M. Sivasamy[2] and M. K. Menon[2]** ([1]Division of Genetics, Indian Agricultural Research Institute, [2]IARI, Regional Station, Wellington, India)
Transfer of rust resistance genes in Indian wheat cultivars and their deployment in different agro-ecological zones

The wheat crop in India is attacked by several diseases. Amongst, rusts (*Puccinia* spp) are prominent and causes substantial losses during epidemic years. Leaf rust is the most important widely distributed and frequently occurring diseases in the Indian sub-continent. It spreads both from southern and northern hills to the plains. Usually, life span of a wheat variety is shortened by the evolution of new virulent rust pathotypes. Therefore, development of wheat cultivars carrying diverse rust resistance genes with a broad range of effectiveness and their geographical deployment, depending on the race flora, is essential. Introgression by backcrossing method of the effective alien genes, viz. *Lr19, Lr24, Lr28, Lr32* and *Lr37* into well adapted commercial Indian bread wheat (*Triticum aestivum* L.) cultivars, which are highly susceptible to leaf rust was the principal objective of the study. The *Secale cereale* segment carrying linked genes *Sr31 Lr26 Yr9 Pm8* was also introduced into six popular Indian cultivars, namely, HD2329, WH147, Lok-1, HUW234, C306 and NI5439 and the improved lines were recombined with three leaf rust resistance genes, *Lr24, Lr28, and Lr32*. Some of the backcross lines carrying alien genes have been released for commercial cultivation in India.

S-3-4 **H. S. Dhaliwal[1], P. Chhuneja[2], K. Singh[2]** ([1]Indian Institute of Technology, Roorkee, [2]Punjab Agricultural University, India)
Wild germplasm of wheat –a mine of variability for food and nutritional security–

The genetic bases for resistance against various wheat diseases among bread wheat cultivars of India is very narrow. Breeding wheat for superior bread making quality and high nutritional quality has not been seriously taken up. To ensure adequate food and nutritional security for the ever-increasing population not only more wheat grains with higher nutritional quality have to be produced from less area but also the production at higher level of productivity is to be sustained against biotic and abiotic stresses. Germplasm of related wild progenitor and no-progenitor *Triticum* and *Aegilops* species constitute a very rich reservoir of useful variability for breeding for high nutritional quality and resistance against biotic and abiotic stresses. Under a series of collaborative USDA funded projects the germplasm of various *Triticum* and *Aegilops* species was collected, maintained and screened under field and laboratories conditions for resistance against diverse pathotypes of various diseases of wheat including rusts, Karnal bunt, powdery mildew and cereal cyst nematode. High level of useful variability was discovered for resistance against various diseases and other traits both within and among species. About 20 genes with different mechanisms for resistance against various diseases were transferred from eight species through interspecific hybridization with or without induced homoeologous chromosome paring, molecular

cytogenetics and molecular markers. Suppression of resistance was found to be ubiquitous phenomenon in interspecific crosses. The *Ph*[1] gene of *Aegilops speltoides* was found to be highly satisfactory for induced homoeologous chromosome pairing. Transfer of high molecular weight glutenin subunits from wild *Triticum* species into *T. durum* not only increased the protein content but also improved the sedimentation value of the recipient line. Pyramiding of leaf rust resistance genes using marker assisted selection in the elite wheat cultivars is in progress. Variability for three to fold higher level of iron and zinc content in the grains of wild A, D and S genome species compared to that of durum and bread wheat cultivars has been found which can be used for overcoming the hidden hunger for the micronutrients in half of the world population. Some of the introgression derivatives with useful variability have been registered with the National Bureau of Plant Genetic Resources, New Delhi for use at the international level. Recent advances of molecular breeding, comparative and functional genomics, gene cloning and genetic transformation can be used for reducing linkage drag, gene discovery and allele mining for efficient and effective utilization of the useful variability for wheat improvement

Poster presentation

P-1 **R. Iwasaki[1], J. Mozafari[2], S. Ohta[1]** ([1]Department of Bioscience, Fukui Prefectural University, [2]National Plant Gene-Bank, Iran)
A brief report on the cultivated, weedy and wild rye collection in Iran by a Japanese-Iranian cooperative field research project

Fukui Prefectural University and National Plant Gene-Bank of Iran carried out cooperative field researches in 2003 and 2004 in the northern and eastern parts of Iran, one of the diversity centers of rye. They surveyed the Elburz Mountains, the northern part of the Zagros Mountains and the southern coastal regions of the Caspian Sea. 82 samples of *Secale cereale* with brittle and non-brittle spikes were collected at 58 sites, and 9 samples of *S. montanum* were collected from its natural populations. We report the geographical distribution of the collection sites and the frequency of the plants with brittle spikes in each *S. cereale* population. This project is financially supported by the Japanese Society for the Promotion of Science (Grant-in-Aid No.15255012).

P-2 **M. Niwa[1], H. Ozkan[2], S. Ohta[1]** ([1]Department of Bioscience, Fukui Prefectural University, [2]Faculty of Agriculture, Cukurova University, Turkey)
Distinct difference in seed dormancy among the grains on different florets of the same spikes in some *Aegilops* species

A field observation at natural populations of some *Aegilops* species revealed a distinct difference in seed dormancy between the grains bearing on first florets and those on second florets of the same spikes. The latter germinated soon after shattering of spikes, while the former showed deeper dormancy and often germinated in the next year of shattering. Germination in a total of 63 accessions of four *Aegilops* species was examined under a controlled condition. The result confirmed the observation at natural populations. This study is a part of the cooperative research project between Fukui Prefectural University, Japan and Cukurova University, Turkey, and is financially supported by the Japanese Society for the Promotion of Science (Grant-in-Aid No.15255012).

P-3 **T. Hoshino[1], Oleg B. Tkachenko[2], I. Yumoto[3], N. Matsumoto[4]** ([1]New Energy and Industrial Development Organization, [2]Main Botanical Garden, Russian Academy of Sciences, [3]National Institute of Advanced Industrial Science and Technology (AIST), [4]National Institute for Agro-Environmental Sciences)
Geographical distribution and mating characteristics of snow mold, *Typhula ishikariensis* in Eurasia

Speckled snow mold, *Typhula ishikariensis* (basidiomycetes) is the important psychrophilic fungal pathogen of winter cereals in the Northern Hemisphere. This fungus is distributed in Japan, Europe and North America. However, there is no report of the geographical distribution in central part of Eurasia (ex. Siberia). We surveyed this fungal distribution in Russian Far East and Siberia, and *T. ishikariensis* was widely distributed in Russian Far East and maritime climate of Siberia (however, this fungus was not found in continental climate in Siberia; ex. Yakutsk). This fungus was divided into two subgroups (biological species I and II; BSI and BSII) from mating characteristics. BSI was widely distributed in

Eurasia, however, BSII was not found from central Siberia to Baltic countries.

P-4 **T. Sasanuma** (Kihara Institute for Biological Research, Yokohama City University)
Preliminary analysis for genetic variation of Japanese indigenous *Elymus* species, *E. tsukushiensis* (Japanese vernacular name Kamojigusa) and *E. humidus* (Mizutakamoji)

Japanese indigenous *Elymus* species is one of the potential genetic resources for Triticeae crops because of their high adaptability for Japanese moist climate and close relationship to the important crops. In spite of such potential importance, the genetic information about these species is very little. To provide the basic genetic information on these species, I conducted preliminary genetic variation analysis of two hexaploid *Elymus* species, *E. tsukushiensis* and *E. humidus*, using several SSR markers and CAPS of a chloroplast region. Samples were collected from four sympatric populations of these two species at Kanagawa, Gifu, Okayama and Miyazaki. With SSR markers intrapopulational variation was detected for both species. The chloroplast CAPS suggested the possibility of introgression between the two *Elymus* species.

P-5 **S. Konishi, Y. Nemoto, T. Sasanuma, T. Sasakuma** (Kihara Institute for Biological Research, Graduate School of Integrated Science, Yokohama City University)
The structure and expression of *Mlo* genes in wheat

The *Mlo* gene is encoding a plant specific protein containing seven trans-membrane domains. By searching on the database, seven *Mlo* genes of wheat including known gene *TaMlo1* were identified as ESTs. The phylogenetic analysis of the *Mlo* family members originated from several monocot and dicot species revealed that the *Mlo* genes had been generated and diverged at least earlier than monocot-dicot diversification. Gene specific RT-PCR revealed differential expression of *TaMlo* genes in response to treatments with salt or mannitol, in root and shoot. These observations indicate that the expression of the *Mlo* family is regulated by differential mechanisms for each gene and suggest that each of the MLO proteins in wheat may function differently depending on tissues or environmental stresses.

P-6 **S. Kadosumi[1], T. Sasanuma[1], T. Kawahara[2], T. Sasakuma[1]** ([1]Kihara Institute for Biological Research, Yokohama City University, [2]Graduate School of Agriculture, Kyoto University)
Multiple origin of tetraploid species based on U genome-specific CAPS marker U31

Polyploidization is important for plants. To elucidate the relationship of polyploidization with speciation, we revealed the intra- and interspecific variation of the four UUMM genome tetraploid *Aegilops* species (*Ae. ovata*, *Ae. columnaris*, *Ae. triaristata* and *Ae. biuncialis*) and the UU genome diploid *Ae. umbellulata*. Among the 30 primers tested to construct genome specific marker, U31 demonstrated the U genome-specific amplification. The size polymorphism and RFLP with *Msp*I indicated that the U31 locus had four alleles in all the 264 accessions studied. Among them, the major allele and the allele with a 123bp deletion were detected in both the diploid and two of the tetraploid (*Ae. columnaris* and *Ae. triaristata*). This result indicates multiple origin of these two tetraploid species.

P-7 **M. Okuda, J. Kimbara, S. Nasuda, T. R. Endo** (Laboratory of Plant Genetics, Graduate School of Agriculture, Kyoto University)
Chromosomal localization patterns of a heterochromatin protein HP1 in wheat and related species

Heterochromatin is a chromosomal region where chromatin is highly condensed. In yeast, fly, and vertebrates, heterochromatin protein 1 (HP1) plays a key role in heterochromatin formation. In order to characterize the function of the HP1 in plants, we isolated genes encoding for HP1 homologues from hexaploid wheat *Triticum aestivum*. We prepared antibodies against wheat HP1 homologues and investigated localization patterns of the HP1 proteins in mitotic chromosomes of hexaploid wheat and related species by the immunofluorescence microscopy. In diploids, barley, rye, and *T. monococcum*, and in tetraploid *T. durum*, HP1 mainly localized to centromeres. In hexaploid wheat, however, HP1 signals were observed along the length of every chromosome. Our observations suggest that the relocation of HP1 has possibly occurred through polyploidization.

P-8 **T. Kitamura[1], S. Nasuda[1], T. Nomura[1], Y.**

Ogihara[2], T. R.Endo[1] ([1]Laboratory of Plant Genetics, Graduate Schookl of Agriculture, Kyoto University, [2]Laboratory of Genetic Engineering, Graduate School of Agriculture, Kyoto Prefectural University)

Analysis of mutations found in the deletion lines of chromosome 5A of hexaploid wheat

We investigated molecular basis of the RFLPs between normal and deletion lines of Chinese Spring wheat that were found during deletion mapping of a cytochrome P450 gene (*TaBx3*) to sub-arm regions of chromosome 5A. We identified a TAC clone carrying the *TaBx3-A1* allele and designed PCR primers to specifically amplify sequences surrounding the *TaBx3-A1* allele. Comparing the nucleotide sequences, we revealed that the RFLP detected in DraI digestion was caused by a nucleotide substitution, and that nucleotide substitutions were widespread throughout the 58 kb interval. Sequence comparisons of the *TaBx3* genes ruled out the possibilities of homoeologous recombination between group-5 chromosomes and introgression from *Aegilops cylindrica* whose gametocidal chromosome 2C was used for the production of the deletion lines.

P-9 **K. Kawaura[1], K. Mochida[2], Y. Yamazaki[3], Y. Ogihara[1]** ([1]Kyoto Prefectural University [2]Nagahama Institute of Bio-Science and Technology [3]National Institute of Genetics)

Global analysis of gene expression patterns in response to salt stress using wheat 22k cDNA oligo-microarray

For investigating the transcriptome of hexaploid wheat, we have designed cDNA oligo-microarray including 21,939 genes. From 148,676 ESTs, 34,064 contigs were grouped by the phrap method. The sense strand of each contig was predicted and the 60 mers of probes were designed (Agilent Co Ltd). Then we conducted pilot experiments to validate by using common wheat treated with salt. Every experiment revealed that most probes were detectable and reproducible for signal intensities. We selected the 1,811 genes whose expressions were two-fold changed by salt-treatment. These genes were classified according to the hierarchical clustering method and functions of these groups were inferred with the gene ontology. By applying these tools, functions of wheat genes, if not all, might be possibly estimated.

P-10 **M. Yamamoto[1], N. Fujiwara[2], G. Suzuki[2], Y. Mukai[2]** ([1]Department of Nutritional Sciences for Well-being, Faculty of Health Sciences for Welfare, Kansai University of Welfare Sciences, [2]Laboratory of Plant Molecular Genetics, Division of Natural Science, Osaka Kyoiku University)

Physical mapping of LMW glutenin genes in wheat by FISH

In order to analyze the over-all organization of LMW glutenin gene region, we visualized the overlapping region among clones and distribution of the genes by fluorescence in situ hybridization (FISH). We used three BAC clones, I3, D8, and D9, with the large DNA fragments containing the LMW glutenin gene of *Aegilops squarrosa* (2n=14, DD). FISH data showed that the length of the overlapping region between I3 and D8 was 10.5 kb, and 5.4 kb between D8 and D9. But, no overlapping region between I3 and D9 was observed. Three clones were located in order of I3, D8, and D9, spanning 279.1 kb in the LMW glutenin gene region. The FISH experiment using subclones of the gene as probes, visualized that I3, D8, and D9 clones include two, four, and four loci, respectively.

P-11 **C. Shimamura, R. Ohno, C. Nakamura, S. Takumi** (Faculty of Agriculture, Kobe University)

Improvement of freezing tolerance in transgenic tobacco with wheat cold-responsive genes

A wheat cold-responsive gene *Wcor15* encodes a chloroplast-targeted COR protein. Three cold-responsive elements (CRTs) were found in the 5' upstream region of *Wcor15*. The CRT motifs are putatively recognized by a transcription factor WCBF2 in wheat cells. To investigate functions of the *Wcor15* and *Wcbf2* genes under low temperature conditions, we produced transgenic tobacco plants with each of the genes under control either of the CaMV35S promoter or the 5' upstream region of *Wcor15*. Transgenic plants overexpressing the introduced chimeric genes dramatically increased freezing tolerance, clearly indicating their positive contribution in the development of freezing tolerance in plant cells. The WCOR15 protein fused to GFP was targeted into the stroma of tobacco chloroplasts.

P-12 **K. Hori, K. Sato, K. Takeda** (Research Institute for Bioresources, Okayama University)

Quantitative trait loci for BaYMV resistance in barley

Barley yellow mosaic virus (BaYMV), which is

transmitted by the soil-borne fungus *Polymyxa graminis*, causes serious damage on winter barleys in East Asia and Europe. Resistance loci were identified on two recombinant inbred populations derived from Russia 6 x H.E.S. 4 (RI1) and Harbin 2-row x Turkey 6 (RI2), and a doubled haploid (DH) population derived from Haruna Nijo x H602 (*Hordeum vulgare* ssp. *spontaneum*). By the reaction to BaYMV on field in 2003 season, four, five and three QTLs were detected in the RI1, RI2 and DH populations, respectively. The relationship between the QTLs identified in this study and the reported BaYMV resistance genes, described as *rym1* to *rym13* is discussed.

P-13 **H. Nishida, Y. Ohmura, Y. Akashi, H. Yoshino, K. Kato** (Faculty of Agriculture, Okayama University)

Geographical variation in the promotor sequence of a *Vrn-A1* allele in hexaploid wheat

In hexaploid wheat, a *Vrn-A1* allele for spring growth habit has three variations in its promotor region; 20 bp deletion, no in/del (the same as winter allele), and 124 bp insertion, among which the last variation has not been found in tetraploid wheat (Yan et al. 2004). We investigated these variations among 200 wheat landraces, with the *Vrn-A1* allele, collected from various parts of the world. Geographical distribution of each variation suggested the transmission routes as below. The no-in/del type transmitted eastward along the southern foot of the Tibetan highland to the northern part of China and southward to the north-eastern part of Africa, the 20 bp-deletion type transmitted to the north-eastern part of Africa and the Mediterranean region, and the 124 bp-insertion type transmitted to the northern part of China along the Silk road and to the Mediterranean region and Europe.

P-14 **S.K. Ghimire[1], T. Ikeda[2], N. Ishikawa[2], S. Ohnishi[1], Y. Akashi[1], H. Nishida[1], K. Kato[1]** ([1]Faculty of Agriculture, Okayama University, [2] WeNARC)

PCR-based evaluation of genetic diversity in HMW- and LMW-glutenin subunit in wheat

Genetic diversity in high molecular weight (HMW-GS) and low molecular weight glutenin subunit (LMW-GS) was analyzed by PCR-based technique. Among 634 wheat accessions examined, three, five and six alleles were identified at *Glu-A1-1*, *Glu-B1-1* and *Glu-D1-1* locus, respectively. *Glu-A1-1a*, *Glu-A1-1b* and *Glu-A1-1c* were frequently observed in South and East Asia, Europe and Central Asia, respectively. Among the alleles of *Glu-B1-1*, *Glu-B1-1a* was frequent in all areas analyzed, while *Glu-B1-1d* was frequent in England and France. New allele at *Glu-D1-1* locus was mainly found in West Asia. *Glu-1D-1e* was frequently observed in Japanese accessions, and also in two and one accessions from Afghanistan and Korea, respectively. We amplified type VI genes of *Glu-A3* and type II genes of *Glu-B3* for LMW-GS after Ikeda et al. (2002), and five and three bands with different size were observed, respectively. 1240 bp type was frequently found in Europe, and its frequency decreased in East Asia where 1320 bp type was frequent. Similar geographical cline was observed also in *Glu-A3*. PCR products with different size were sequenced for both *Glu-A3* and *Glu-B3*, and compared with the known sequences.

P-15 **H. Tanaka, R. Shimizu, H. Tsujimoto** (Faculty of Agriculture, Tottori University)

Contribution of low-molecular-weight glutenin subunits to dough strength in common wheat (*Triticum aestivum* L.)

To analyze the effect of low-molecular-weight glutenin subunit (LMW-GS) genes on dough strength, locus-specific primers of LMW-GS genes and gliadin bands tightly linked to LMW-GS genes were used in common wheat. Segregation analysis of the F_2 progeny from a cross between Haruhikari, a good bread-making quality cultivar, and Asakaze-komugi, a poor bread-making quality cultivar, showed that the significant effect on dough strength was concerned with one amplified LMW-GS gene located on *Glu-B3* locus from Haruhikari. The LMW-GS gene of Haruhikari relative to Asakaze-komugi had a seven amino-acid deletion in the repetitive domain, and three amino-acid substitutions caused changes of hydrophilicity. The presence of the LMW-GS gene and other LMW-GS genes tightly linked to it may affect the dough strength.

P-16 **A. A. Hagras[1], M. Kishii[2], K. Sato[3], H. Tsujimoto[1]** ([1]Faculty of Agriculture, Tottori University, [2]CIMMYT, [3]Research Institute of Bioresources, Okayama University)

Extended application of barley expressed sequence Tag (EST) markers for the genomic analysis of wheat and barley-related species

The applicability of three series of EST-based-PCR primers of barley to ten wild species covering wide range of variation in Triticeae was examined in this study. The first series were randomly chosen from the pool of barley EST primers, while the other two series were pre-screened and showed polymorphic fragments between barley and wheat in the previous study. The percentage of primers that produced single clear band in the studied species ranged from 40 to 76%. The frequency of amplification and the polymorphism agreed well with the phylogenetic distance from barley. Number of the amplified polymorphic markers using the pre-screened primer sets was significantly higher than that of using the randomly chosen ones. A large number (79-572) of DNA markers for the different alien species used in this study were obtained from the 1,165 EST-based-PCR primers of barley examined. These markers will be valuable tool in identifying the alien chromosomes added to wheat genome.

P-17 **Y. Saito[1], M. Kishii[2], T. Abe[3], H. Tanaka[1], H. Tsujimoto[1]** ([1]Faculty of Agriculture, Tottori University, [2]CIMMYT, [3]Riken)

Chromosome engineering in wheat by heavy ion beam irradiation

Triticeae species are genetic resources for the improvement of wheat and barley. We have introduced the chromosomes of wild species to common wheat. The next steps necessary to be solved are to find the location of the useful gene and introduce the chromosome segment to a wheat chromosome. Here we demonstrate heavy ion beam as a useful method to break chromosome. We irradiated nitrogen (N) and neon (Ne) ion beams to the dry seeds of common wheat cv. Chinese Spring (CS) and of the disomic addition line with the chromosome J of *Leymus racemosus* (Kishii et al. 2004). The chromosomes were observed by acetocarmine staining and genomic in situ hybridization to the chromosomes in the root tip cells. We found that LD_{50} after one month was 150 and 90 Gy in N and Ne, respectively. Chromosome observation by GISH revealed occurrence of about 20 chromosome breakage by irradiation of 30 Gy of Ne ion beam, which is eight times higher than the value observed by acetocarmine staining.

P-18 **M. Ito, M. Kishii, A. A. Hagras, H. Tanaka, H. Tsujimoto** (Faculty of Agriculture, Tottori University)

Production of *Psathyrostachys huashanica* chromosome addition lines and finding its use for breeding.

Psathyrostachys huashanica Keng (2n=2x=14,NhNh) is a perennial wild species distantly related to wheat. It is endemic in Huashan branches of Qinling mountains in China. It has yellowish green thin leaves and 40–60 cm in height, early-maturing, drought and poor soil tolerance and resistance to many diseases such as stem rust, leaf rust, black rust, and powdery mildew. It is also known to be resistance to damping-off that is one of the obstacles to continuous cropping of wheat. We crossed wheat (*Triticum aestivum* cv. Chinese Spring) with the pollen of *P. huashanica* and produced the chromosome addition lines. We selected monosomic addition line in all chromosomes and are selecting disomic chromosome addition lines from the selfed offspring. We selected *P. huashanica*-specific PCR markers from the barley EST markers developed by Sato et al. and identified the added chromosomes.

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V. Announcement

Publication of a 100th memorial issue of WIS

Wheat Information Service (WIS), published its first issue in October, 1954, will be discontinued with the 100th issue, of which publication is scheduled in 2005, due to financial problem of its supporting body, Kihara Memorial Yokohama Foundation, Yokohama, Japan.

To commemorate its 50 years' history of publication and the 100th issue in the coming year, the Foundation has decided to publish a special issue in a form of book. Its tentative title is "Frontiers of Wheat Bioscience", will be published by the end of 2005.

Dr., Koichiro Tsunewaki, emeritus professor of Kyoto University has accepted to take his initiative to edit the memorial issue. His idea is to edit this book with invited articles prepared by active researchers in the fields of wheat genetics and breeding, who are expected, from a worldwide viewpoint, to play a key role in progress of these fields for future decades. He asked recommendation of such persons to the International Advisory Board members of WIS.

Based on their recommendation and his own thought, he chose 16 wheat researchers, inviting them to contribute their original articles.

Tentative content and plan of this memorial book are as follows:

Title of book: "Frontiers of Wheat Bioscience" (tentative)
Chapter makeup: Authors and tentative subjects (not title)

1] Genetics of differentiation
   1. Faris, J. D., USDA/ARS Cereal Crops Research Unit, USA: Q-gene for square-headedness
   2. Murai, K., Fukui Prefectural University, Fukui, Japan: Floral differentiation
   3. Byrne, M., John Innes Centre, Colney, UK: Inflorescence architecture

2] Genetics of biotic and abiotic stress response
   4. Lagudah, E. S., CSIRO, Canberra, Australia: Rust and cyst resistant genes
   5. Ma, Zhengqiang, Nanjing Agricultural University, Nanjing, China: Use of alien genetic materials
   6. Takumi, S., Kobe University, Kobe, Japan: Genes for cold tolerance

3] Quality
   7. Rahman, S., CSIRO, Canberra, Australia: Seed softness gene
   8. Porceddu, E., University of Tusca, Viterbo, Italy: Storage protein genes

4] Chromosome study and manipulation
   9. Mukai, Y., Osaka Kyouiku University, Osaka, Japan: ISH in chromosome studies
   10. Dolezel, J., Inst. Experimental Botany, Olomouc, Czech Republic: Flow sorting of chromosomes
   11. Moore, G., John Innes Centre, Colney, UK: Meiosis and Ph1 gene
   12. Tsujimoto, H., Tottori University, Tottori, Japan : Chromosome engineering

5] Genomics
   13. Gill, K. S., Washington State University, Pullman, USA: Genome mapping
   14. Ogihara, Y., Kyoto Prefectural University, Kyoto, Japan: Genomics
   15. Levy, A., Weizmann Institute of Science, Rehovot, Israel: Genome divergence after polyploidization
   16. Somers, D. J., Eastern Cereal and Oilseed Research Centre, Ottawa, Canada: Microsatellite mapping in breeding and genetics

Schedule of publication
   Manuscript submission: April, 2005
   Editing works: up to August, 2005
   Publication: December, 2005

Distribution
Distributed to the registered members of WIS with asking for voluntary contribution to cover mailing and publication cost.

A proposal for e-WIS

Wheat Information Service (WIS) and its Business Office will close in June 2005 with the publication of No. 100 as a commemorative issue. As mentioned in the "Important Announcement on WIS – Discontinuance of Publication" by Dr. Kozo Nishikawa, the Editor-in-Chief, the prime aim of WIS is to promote "exchanging information about wheat genetics and breeding among wheat researchers in the world" (No. 98, 2004). In appreciation of the significant role WIS has played over the last half a century, the community of Japanese wheat geneticists has decided to continue promotion of exchanging information through the World Wide Web. Four scientists, S. Nasuda, S. Takumi, Y. Matsuoka, and T. Sasanuma voluntarily will take part in the editing of the electronic version of WIS (tentatively called "eWIS"). The details of eWIS are now being discussed. The following points have been agreed among the four scientists who will constitute the Editorial Office of eWIS:

(1) The aim of eWIS is to promote communications among wheat researchers.
(2) Submitted materials will be published as *non-peer-reviewed* articles.
(3) eWIS is published *online only* and distributed through the "KOMUGI" web page (UTL, http://shigen.lab.nig.ac.jp/wheat/komugi/top/top.jsp).

Editorial policies and article guidelines of eWIS will appear in the forthcoming issue of WIS and on the web page of "KOMUGI". We welcome comments and suggestions from everybody. Please feel free to write us.

11th December, 2004

eWIS Editorial Office
Shuhei Nasuda (Kyoto University, nasushu@kais.kyoto-u.ac.jp)
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VI. Editorial remarks

This is the last volume of Wheat Information Service (WIS), published in the normal edition. We, editorial board, are glad to have an ideal style of WIS in the present volume, including a review article by Dr. M. Muramatsu, six research articles from five countries, three Research Information, Record and Announcements. The centennial issue will be in a special edition with reviewing papers, chief-edited by Dr. Koichiro Tsunewaki as announced in the previous page of this issue.

It was 50 years ago when 26 wheat scientists had discussed on organizing an international exchange and cooperation program for wheat research on the occasion of the 9th International Congress of Genetics held at Bellagio, Italy in 1953, and reached a conclusion of publishing a research journal, Wheat Information Service. Since then, WIS has been published biannually with together organizing international wheat genetics symposium every 5 years. Worldwide contributors, in a maximum record covering 64 countries with 677 subscribers, have published WIS with supports. The chief editors in its history are; Dr. Kosuke Yamashita (1954-1986), Dr. Masatake Tanaka (1986-1992), and Dr. Kozo Nishikawa (1992-present). Staffs of Kyoto University, Kihara Institute for Biological Research and Kihara Memorial Foundation worked as business managers in these respective terms. Members of International Advisory Board, and Editorial Boards (mostly Japanese scientists) have contributed for continuous publication and for improvement of contents of WIS. WIS has been organized and maintained on the voluntary bases for mutual supports among wheat researchers, although Kihara Memorial Foundation had accepted as the publisher for last 10 years. We should not forget to note our appreciation to Ministry of Education of Japan, Japanese Miller's Association, Kihara Institute for Biological Research, and Kihara Memorial Foundation for their financial supports. Thank to these contributions, research notes and article and information totaling 1,050 have been published during the past fifty years as follows:

Nos.	Years	Articles published	Nos.	Years	Articles published
1-10	1954-1959	155	51-60	1980-1985	92
11-20	1960-1965	108	61-70	1986-1990	96
21-30	1966-1970	109	71-80	1990-1995	98
31-40	1970-1975	102	81-90	1995-2000	115
41-50	1976-1979	104	91-99	2000-2005	71

We are proud that many articles appeared in WIS had originality and are still important, cited by many scientific reports. Also, it should be mentioned that WIS have contributed to wheat scientific world by publishing lists of genetic stocks and catalogues of gene symbols (special thanks to Dr. R. A. McIntosh for his efforts of edition). Back numbers are available on request to Kihara Memorial Foundation.

Due to modern development of information exchange media and financial problem, WIS is going to terminate. However, we hope WIS had contributed to the wheat research, and its idea of free exchange of research information with mutual support will be inherited. Thank you very much, and good luck for wheat research.

January 17, 2005

Editorial Office of WIS
Kozo Nishikawa, Tetsuo Sasakuma, Hisashi Tsujimoto, & Keiko Furukawa

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