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INTRODUCTION

The perennial lymegrass

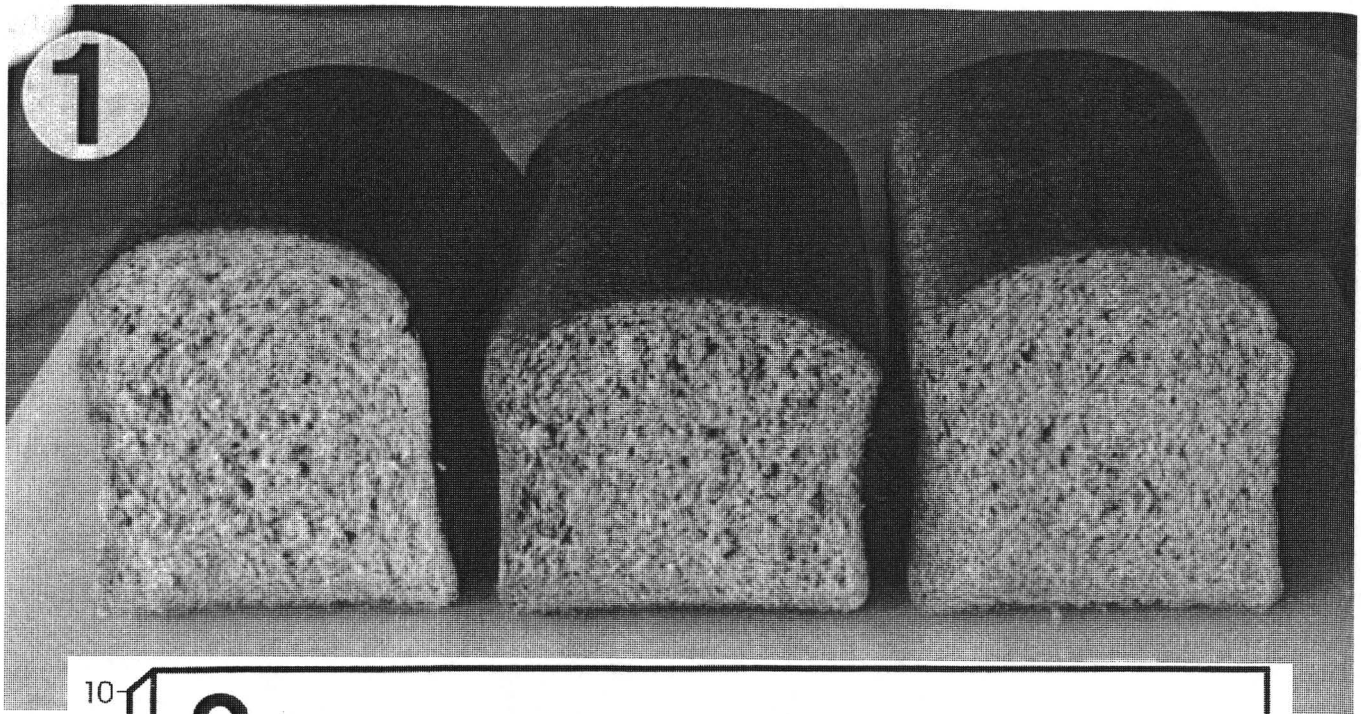
The most common species of lymegrass in the northern circumpolar regions are the European *Leymus arenarius* (L) Hochst. and the American *L. mollis* (Trin.) Pilger (Löve and Löve, 1975). These perennial and rhizomatous species of the tribe Triticeae tend to colonize coastal areas, but inland populations are also found. Morphologically the two species are so similar that it is often difficult to differentiate them, whereas cytologically they have different chromosome numbers. *Leymus arenarius* is octoploid with 2n=8x=56 and *L. mollis* is a tetraploid species with 2n=4x=28 (Löve and Löve, 1948; 1975), but both species share the same basic genome constitution (NX; Wang and Jensen, 1994). These two closely related species are geographically separated: *L. arenarius* is found in northern Europe, from Lapland and north-west Russia, Scandinavia and the countries along the Baltic Sea, to central Europe, and from England, Scotland, Faroe Islands to Iceland; whereas *L. mollis* is found in Greenland and the north American continent, on the shorelines of both the Atlantic and Pacific coasts, in the Canadian Arctic and Alaska (Sigurbjörnsson, 1960; Barkworth and Atkins, 1984). The species also coexist in some places due to natural or intentional introductions, for example *L. arenarius* in southern Greenland and Canada (Ahokas and Fredskild, 1991) and *L. mollis* recently introduced in Iceland. The significance of such extensive distribution is that the potential cultivation areas for lymegrass, given domestication, are enormous. At present most of these areas are not suitable for common crop species like wheat or barley.

The aim of the present study is to improve the perennial lymegrass (*L. arenarius* and *L. mollis*) for cultivation as potential cereal crop for Iceland as well as for other regions of native lymegrass distribution. The study will also provide cereal breeders with broader genetic resource containing several characters of the wild species such as tolerance to extreme environments and perhaps resistance to pathological diseases.

The use of lymegrass for bread-making

Lymegrass has a long history of use for human consumption. Earliest records of lymegrass in Iceland date back to the Icelandic sagas. Carbonized remains of the grass have been discovered in Viking archeological sites, especially in Iceland and Greenland, as well as an increase in *Leymus* pollen with the Viking homesteads in Newfoundland (see Griffin and Rowlett, 1981). In Iceland, lymegrass (*L. arenarius*) grains were used as a source of bread flour until the 19th century, and in the south coast areas of Vestur-Skaftafellsssla the local production of lymegrass flour was apparently sufficient that no other flour was imported (Sigurbjörnsson, 1960). Grains of *L. mollis* were also used by North-American Indians, while these of *L. arenarius* were sometimes gathered in north Russia for the same purpose (in Klebesadel, 1985).

The quality of lymegrass flour for bread-making was known to be high, and some reported that products made out of lymegrass flour were even better than from any imported flour at that time (e.g. in Hooker, 1813). But the characteristics of bread-making are unknown by the present standards. In collaboration with the Flour Milling and Baking Research Association at Choleywood (UK), we investigated the quality of lymegrass flour. Lymegrass grains were obtained from Eyrarbakki population in south



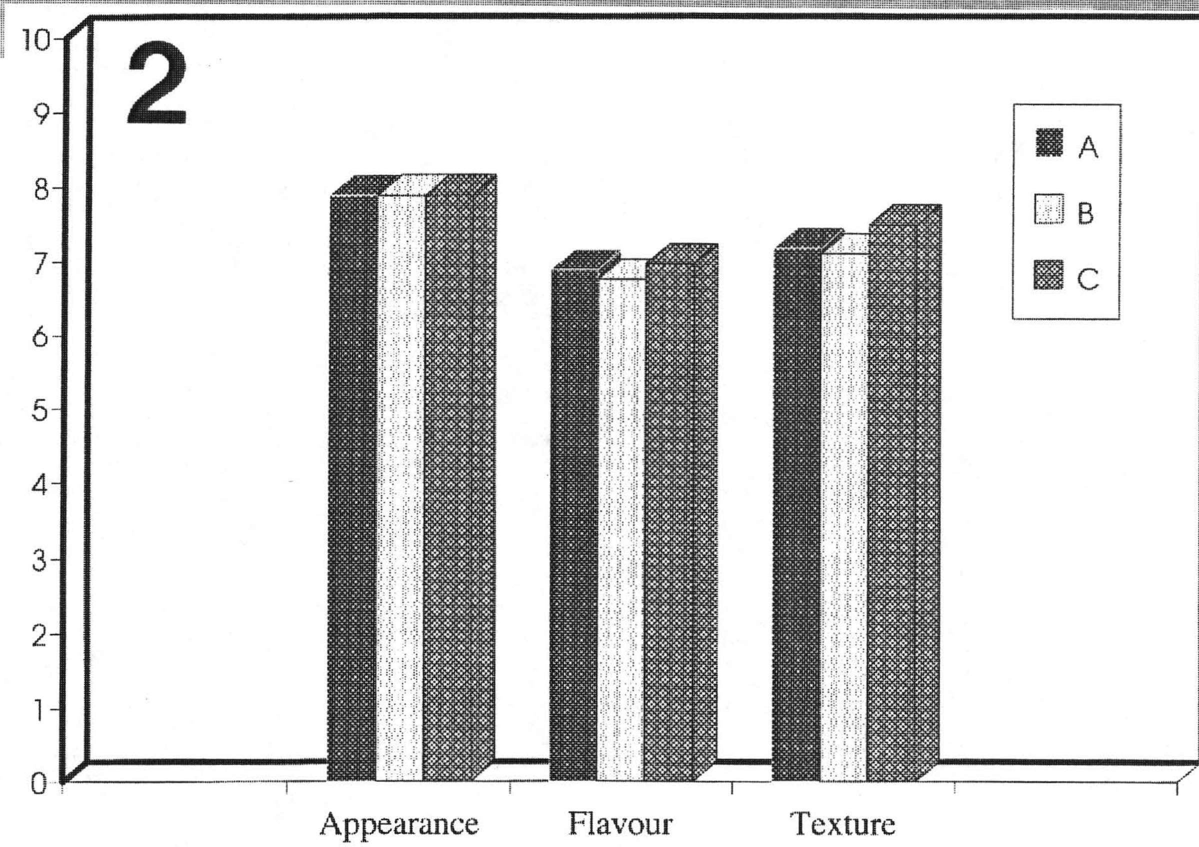


Figure 1. Bread made from different mixtures of whole-meal wheat flour from Canadian winter wheat variety and lymegrass flour milled from grains of *Leymus arenarius* havested in Eyrarbakki, south Iceland: (A) 100% wheat flour, (B) 85% wheat flour and 15% lymegrass flour, and (C) 80% wheat flour, 15% lymegrass flour and 5% gluten. The baking was prepared by Flour, Milling and Bread Research Association at Choleywood, UK.

Figure 2 Results of taste test of the bread in Fig. 1, conducted by Food Research Department, Icelandic Agricultural Research Institute. the evaluation was given as values from 9 to 10, where 0: unacceptable, 5: neither good nor bad, and 10: exceptionally good. The means of 25 independent evaluations are presented here. The standard deviation for all three characters is small, between 1.0 and 1.5.

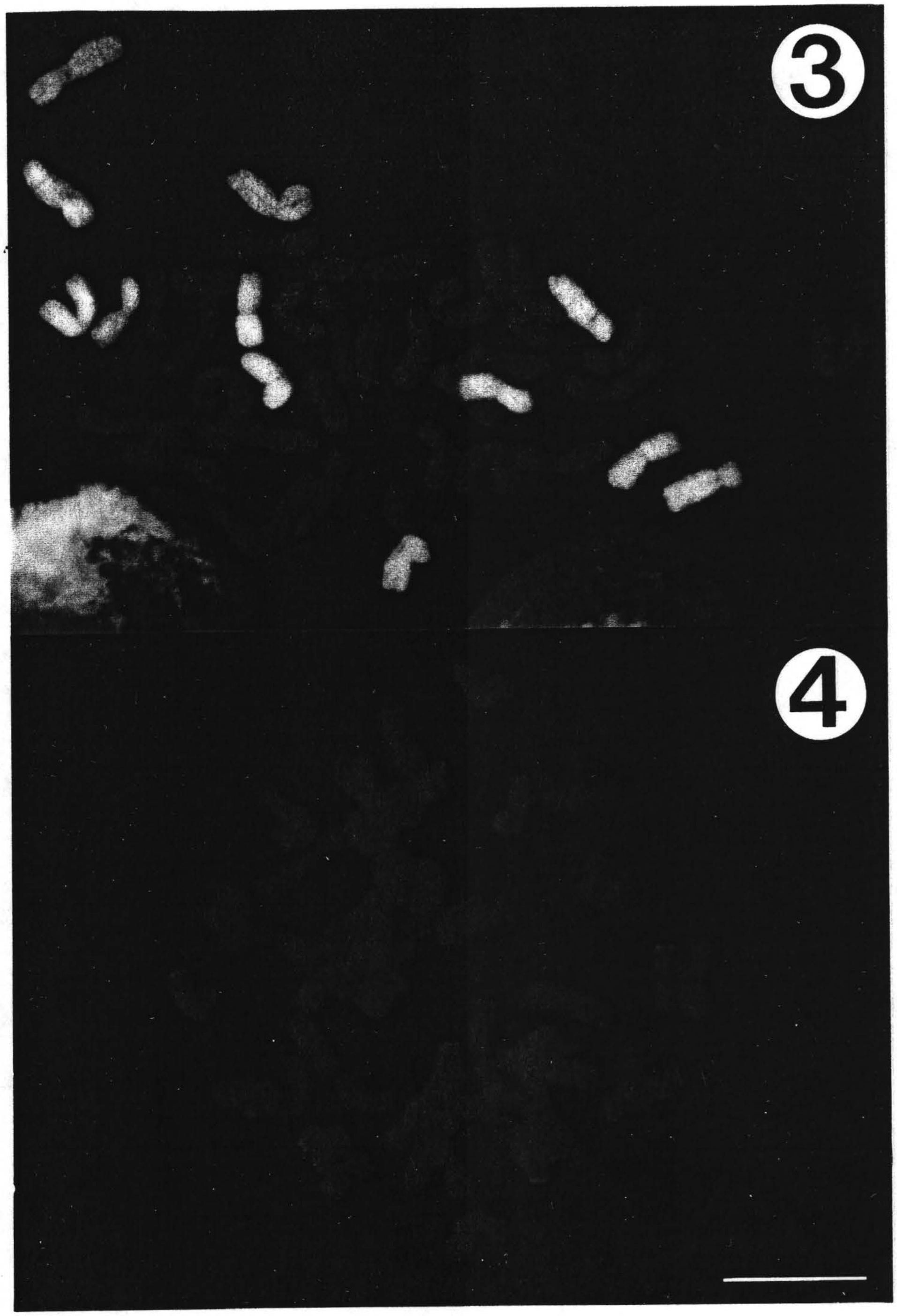


Fig. 3. A metaphase cell of wheat x lymegrass partial amphiploid PI442574 showing 12 yellow-green FITC fluorescing chromosomes originated from lymegrass and 30 red propidium iodide stained wheat chromosomes.

Fig. 4. A metaphase cell of wheat x lymegrass hybrid (*Triticum aestivum x Leymus arenarius*) showing a haploid set of 28 green FITC fluorescing lymegrass chromosomes and a haploid set of 21 red-brown rhodamine fluorescing wheat chromosomes. Scale bar: 10 μm.

Iceland. The grains were mechanically harvested and threshed, using the facilities developed for seed production for land reclamation purposes (Greipsson and Davy, 1994). Icelandic lymegrass (*L. arenarius*) has relatively large grains, about on third to half the dry weight of wheat grains, depending on accessions, and are twice as large as most samples of *L. mollis*. Whole grains were milled and the flour was used in the baking experiment as described in Fig. 1. Three different breads were made: (A) all wheat bread. (B) wheat bread containing 15% lymegrass and (C) wheat bread containing 15% lymegrass and 5% pure gluten, each bread in triplicate. Some of the results have been obtained and among them are the taste testing (Fig. 2) and chemical analysis of the breads. The breads made from wheat and lymegrass flour mixtures (B and C) were similar to that of the high quality wheat bread (A), in appearance, flavor and texture (Fig. 2). In general, all three breads were highly acceptable. In addition, the breads containing lymegrass flour were described as having favorable characters as nut-like flavor and yellowish color. The bread made from wheat and lymegrass flour mixture (B) was more flat than the control bread (A), but when supplemented with pure gluten (C), its physical quality was recovered. However, all the breads had high protein content and good nutritional and dietary quality. The high protein content was found in both the wheat and the lymegrass flour. The wheat variety used in this experiment has exceptionally high total protein (17% dry weight) and lymegrass flour contains about 19%, whereas flour of most wheat varieties contains between 9% and 14% protein (Reykdal, 1993). The present study also shows that the lymegrass flour has significantly higher mineral content, especially calcium, potassium and iron, than all other cereal flour, while its fatty acid content is lower than in other cereals. The overall quality of lymegrass flour, however, appears to be variable among the accessions and further studies will be important for selection of the breeding materials.

BREEDING

Wide-hybridization for simultaneous improvement of lymegrass and wheat

Bread wheat (*Triticum aestivum* L. em. Thell.) is used in the wide-crossing program aiming to transfer important crop characteristics into lymegrass, for example physical quality of bread-making and grain size. Wheat and lymegrass wide-hybrids have been made, from both *L. arenarius* and *L. mollis*. The hybrids will be used for developing amphiploids and for back-crossing with the lymegrass parents, aiming to produce lymegrass breeding lines containing crop characters of wheat while maintaining characters of the wild species such as perenniality and adaptability to sub-Arctic environments.

The wide-hybrids can also be used for wheat improvement. In contrast to the new breeding program for lymegrass described here, transfers of characters from wild species to crops have been extensively practiced (e.g. Gale and Miller, 1987). Several Triticeae species have been involved in wheat improvement - for example, rye (*Secale cereale*) in wheat cultivars 1B/1R (reviewed in Heslop-Harrison et al., 1990), wild barley (*Hordeum chilense*) adding nematode resistance to wheat (Person-Dedryver et al. 1990) and *Agropyron* for rust disease resistance (Friebe et al., 1992). *Leymus*, especially Asiatic species like L. *racemosus* and L. *multicaulis*, has also received much attention for wheat breeding (Mujeeb-Kazi and Rodriguez, 1981; Dong et al., 1986; Plourde et al., 1989), and wheat breeding lines containing *Leymus* chromosomes have been identified. Several traits of *Leymus* have been targeted, especially the resistance to virus and fungal diseases. The relevance of the present study to wheat breeding is that the sub-Arctic lymegrass species (i.e. *L. arenarius; L. mollis*), which has been little exploited, can add to the genetic diversity of crops via the wide-hybrids wheat x lymegrass.

Wide-hybrids involving wheat (both tetraploid and hexaploid species) and several species of *Leymus* were made in the early 1960's (e.g. Tsitsin, 1965; D. Dewey, unpublished), and a few amphiploid lines deriving from these hybrids are still maintained. We have obtained two partial amphiploid lines for cytogenetic and breeding purposes: "AD99" from Professor Arnulf Merker, Swedish University of Agriculture at Uppsala, Sweden, and "PI442574/Dewey" from Professor Bikram Gill, Wheat Genetics Center, Kansas State University, USA. The AD99 is derived from back-crossing of the hybrid *T. durum x L. mollis* to bread wheat, while the PI442574 is derived from a cross between a *Triticum* species and *L. arenarius*. Both lines have been maintained for more than ten generations. We found that both amphiploids had 42 chromosomes, 12 of which have originated from *Leymus*, while 30 chromosomes have wheat origin (Fig. 3). One of these lines, AD99, was shown to have high resistance to mildew and leaf rust (Fatih, 1983; Merker, 1992). Although these materials are not suitable for Icelandic climates, they are valuable for genetic studies and breeding. Methods including *in situ* karyotyping and chromosome mapping will allow identification of chromosomes carrying important agronomic characters, while the plant materials can be used for further back-crossing to either lymegrass or wheat.

We made new wide-hybrids between wheat and lymegrass in the summer of 1993. The seed parent used in the wide-crossing was hexaploid bread wheat *T. aestivum* cv. Sicco (CS/5B) provided by Cambridge Laboratory, John Innes Center, Norwich, UK. The pollen parents were the tetraploid American lymegrass *L. mollis* originating from Alaska Peninsula and the octoploid European lymegrass *L. arenarius* collected from a wild stand in Reykjavík. The crosses were conducted in an unheated glasshouse at Korpa Experimental Station in Reykjavík. The method of crossing and embryo rescue followed Laurie and Bennett

(1986). About 3% of the developed ovaries contained embryos. The hybrids were treated with colchicine and the regenerated plants have been grown to flowering. The mature hybrids showed vigorous vegetative growth and rhizomatous habit. Cytological study confirmed the hybridity and the colchicine doubling of chromosomes. Root-tip chromosomes were analyzed using genomic *in situ* hybridization (Schwarzacher it al., 1989; Anamthawat-Jónsson et al., 1990), which was modified using pre-annealing of two differently labeled genomic DNA probes (Anamthawat-Jónsson et al., unpublished) and rapid *in situ* hybridization protocol (Reader et al., 1994). All hybrids before colchicine treatment showed haploid chromosome number of wheat and lymegrass genomes - *T. aestivum x L. mollis*, 5x-35; *T. aestivum x L. arenarius*, 7x=49 (Fig. 4), where 21 chromosomes originated from wheat and 14 or 28 chromosomes from *L. mollis* and *L. arenarius* respectively. No elimination of chromosomes were observed. Colchicine treated plants showed high proportion of diploid root-tip cells. Molecular cytogenetic studies will be important in the further breeding work, especially to follow chromosome behavior and recombination during the stabilization of amphiploids and to identify transfer of chromosomes carrying genes of interest during the production of lymegrass breeding lines.

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