1. Introduction

Barley (*Hordeum vulgare* L. subsp. *vulgare*) is one of the earliest domesticated crop plants (Zohary and Hopf 1993). The genus *Hordeum* comprises over 32 species, including diploid and polyploidy, perennial and annual types, which are spread throughout the world (Bothmer et al. 1991). In terms of acreage and production worldwide, barley is the fourth most important cereal after wheat, rice and maize. In the year 2005, the global barley production was estimated over 137 million tones harvested from 56.19 million hectares (FAOSTAT 2006). Barley is adapted to a broad range of agro-ecological environments and it is tolerant to soil salinity, draught and frost to a considerable level. The crop successfully grows in the arid climates of Sahara, the Tibetan plateaus, the highlands of Himalayas, the mountains of Ethiopia or Andean countries, and the tropical plains of India. The early spring types grow within the Arctic Circle, farther north than any other cereal (Poehlman 1979).

1.1 Origin and domestication

Indications from the archaeological remains at various sites in the Fertile Crescent suggest that barley was domesticated about 10,000 yeas ago in that region along with other crops, e.g., emmer and einkorn wheat, that led to the foundation of the old world agriculture (Zohary and Hopf 1993). The domestication of barley is assumed to have taken place from two-rowed wild barley *Hordeum vulgare* L. subsp. *spontaneum* in the Near East (Harlan and Zohary 1966). However, this not a consensus theory of barley origin, and evidences suggesting alternative ways of barley domestication have been reported (Tanno et al. 2002; Molina-Cano et al. 2005). The controversies surrounding the origin of cultivated barley in the last centuries can be summarized: (1) the six-rowed barley in the Oriental region derived from the six-rowed wild barley, *H. agriocrithon* (HA); (2) the two-rowed barley in south-west Asia and elsewhere originated from the two-rowed wild barley, *H. spontaneum* (HS) and (3) the numerous other forms are either direct descendents of one or other ancestral forms (HA or HS), or derived from hybridization between the two ancestral forms (Li et al. 2004). With the development and advancement of molecular markers in recent years, more precise information on origin and domestication history of barley is emerging. Bdar et al. (2000) demonstrated a monophyletic nature of barley origin based on allele frequency at 400 polymorphic

AFLP loci studied in a world collection of wild and cultivated barley, and showed that the Israel-Jordan area in the southern part of the Fertile Crescent has the highest probability of being the geographical area within which wild barley (HS) was domesticated. The hypothesis of monophyletic origin of barley is further supported by Li et al. (2004), who analyzed the rDNA polymorphism in wild barley accessions derived from Tibet and other parts of the world. It was revealed that the magnitude of genetic diversity of Tibetan wild barleys (HS and HA), which are considered to be the progenitors of the cultivated barley in the Oriental region (Åberg 1940; Xu 1982; Shao et al. 1982), is considerably low which is not sufficient to account for the vast diversity of cultivated barley within the region. Because of the low level of genetic diversity of wild barleys (HS and HA), and the allele distribution patterns at two rDNA loci, i.e., *Rrn1* and *Rrn2*, in wild and cultivated forms of barley (Saghai Maroof et al. 1990; Li et al. 2004), Tibet is unlikely a center of origin of cultivated barley. Moreover, it has been reported that the six-rowed wild barley (HA) found in Tibet may be a hybridization product of two-rowed wild barley (HS) and six-rowed cultivated barley (Tanno and Takeda 2004).

However, Molina-Cano et al. (1999) suggested barley domestication could have taken place outside the Fertile Crescent, particularly in Morocco. This proposition however, was not substantiated by the RAPD analyses of wild and cultivated barley samples derived from the western Mediterranean basin including Morocco (Blattner et al. 2001), and the authors concluded in favor of a monophyletic origin of barley. In contrast to this, Tanno et al. (2002) based on DNA sequence analysis at a marker closely linked to the *vrs1* locus (row-type gene), and more recently, Molina-Cano et al. (2005) with chloroplast SSRs analysis, have shown strong evidences that cultivated barley may have multiple origins. The latter authors proposed Ethiopia and the western Mediterranean as possible centers of barley origin. It is now generally accepted that *H. spontaneum* is the progenitor of cultivated barley, however, it is not clear whether cultivated barley is of monophyletic origin or the domestication events happened in other parts of the word besides the Fertile Crescent.

1.2 Barley genome

The DNA content of *Hordeum* species ranges from 6.85 to 10.67 pg in diploids (2n=14) and up to 29.85 pg in hexaploid species (2n=42) (Jakob et al. 2004). The cultivated barley is a self-pollinating diploid species (2n=2x=14) with a genome size

of approximately 5.3 x 10^9 bp equivalent to 5.5 pg DNA of a haploid nucleus (Bennett and Smith 1976). The barley genome consists of a complex mixture of unique and repeated nucleotide sequences, and approximately 10 to 20 % are tandem arranged repeated sequences while 50 to 60 % are repeated sequences interspersed among one another or among unique nucleotide sequences (Rimpau et al. 1980). The interspersed *copia*-like retrotransposon *BARE*-1 comprises almost 7 % of the barley genome (Manninen and Schulman 1993).

1.3 Barley cultivation and utilization

1.3.1 Global scenario

The largest area under barley cultivation is in Europe (ca. 28.7 million ha) and Asia (ca. 12.24 million ha). The barley acreage in other parts of the world is significantly lower than in these two continents, e.g., North and South America account for about 6.45 million ha, Africa 4.89 million ha, and Oceania about 3.86 million hectares. About 44 % of the world barley production is contributed by the top five barley producing countries that are Russia, Canada, Germany, France and Ukraine, respectively (FAOSTAT 2006). Barley grains are used as human food, to feed farm animals and for malt production which in turn is used to make beer, whisky or other processed food products. In Japan, barley grains are used for special preparations, e.g., barley tea, shochu, miso and as a rice extender (Kays et al. 2005). In the Western world barley is becoming less important as a human food, and it is mainly used to feed farm animals or for malt production. On the other hand, in the highlands of Tibet, Nepal and Ethiopia, in the Andean countries, and also in some areas of North Africa, China and Russia, barley is still an important human food. Because of its low demand as a human food and its lower yield potential compared to other cereals like wheat and maize, the barley acreage in the major barley producing countries is decreasing.

However, barley is a high value crop in large parts of arid and draught inflicted regions (Fertile Crescent region), the Tibetan plateau and the Himalayas, the marginal areas of many developing countries, and Ireland, Scotland and the Nordic region of Europe (Denmark, Finland, Norway and Finland), where the agricultural activities are restricted by a very short vegetation period (Ortiz et al. 2002; Fischbeck 2002). In recent years, barley is becoming an important food grain for human consumption due to its nutritional and clinical values (Bathy 1999; Gill et al. 2002).

Diets containing barley are effective in lowering blood cholesterol in hypercholesterolemic people with a higher risk of cardiovascular diseases (Behall et al. 2004). More recently, whole grain barley and barley containing products have been allowed to claim that they reduce the risk of coronary heart diseases by the US Food and Drug Administration (FDA, http://www.fda.gov). The nutritional and clinical importance of barley foods and public consciousness regarding quality of daily diet, i.e., cereal diversification, may have a positive impact on the demand of barley as a human food in the future.

1.3.2 Barley in the highlands of Nepal, Himalayas

Barley is an important cereal crop in the northern highlands of Nepal along the Himalayas-range. The importance of the crop increases with increasing altitudes towards the North, where other cereals can not be grown successfully. A typical pattern of distribution of hulled and hulless barley exists in this region, i.e., hulless types are frequent in higher altitudes in the North, predominantly above 2,500 (m). The total barley area in Nepal is estimated about 30,000 hectares; however, specific data on hulled and hulless barleys are not available.

Figure 1 Crop production in the upper basin of river KaliGandaki (Kagbeni) in the Himalayas of central Nepal

The important barley cultivation areas in Nepal are the trans-Himalayan valleys that are extended on to the Tibetan plateau. This includes the historical Mustang and Manang valleys that represent the upper basins of the river KaliGandaki and

Marshyangdi, respectively, which are north to the main Himalayas crest in central Nepal. The archaeological evidences indicate that barley was cultivated in this region as early as in the 1st millennium B.C. (Knörzer 2000). In the highlands of the Himalayas, barley is used in different of ways, e.g., grains are consumed as human food, to feed farm animals and to prepare alcohol. Besides this, barley grains are used for medicinal and religious purposes by the ethnic people. The dry biomass after the harvest is stored and used as fodder during off-seasons.

1.4 Hulled *vs* hulless barley

Hulless or naked barley (*H. vulgare* L. subsp. *vulgare*) differs from hulled barley by the loose husk cover of caryopses that is easily separable upon threshing in contrast to hulled barley. The hulless grain character is controlled by the single recessive gene '*nud*' located on the long arm of chromosome 7H (Kikuchi et al. 2003). The domestication of naked barley is believed to occur after the hulled type around 6500 B.C. (Zohary and Hopf 2000). Taketa et al. (2004) suggested a monophyletic origin of naked barley as a single mutation event either from wild barley (*H. vulgare* subsp. *spontaneum*) or from domesticated hulled barley (*H. vulgare* subsp. *vulgare*).

Figure 2 School children of an ethnic community living in the high altitude Himalayas, displaying hulless barley heads (Sharma et al. 1991)

The cultivation of naked barley is less common worldwide than hulled barley. Its distribution is skewed towards East Asia, namely to the Himalayas (Nepal, Bhutan and Tibet), China, Korea and Japan where it accounts for up to 95% of the domesticated barley in some areas (Takahashi 1955; Sun and Wang 1999). Besides East Asia, it is cultivated in Ethiopia at a low frequency (Assefa and Labuschagne 2004). The cultivation is rare in the Western world (Europe, North America) and in Australia where hulled types are prevalent. Hulless barley is mainly used as animal feed; however, it is an important human food in the Himalayas and in Ethiopia.

1.5 Trends in barley breeding

In the last 50 years the yield potential of barley has been tremendously improved in Europe through breeding efforts (Grausgruber et al. 2002; Ortiz et al. 2002). This is due to the development of high yielding cultivars with reduced lodging and improved disease resistance together with improved fertilization and efficient production technology. The breeding methodologies used in this period are intensive selection in local landraces followed by cycles of cross breeding which first made use of hybridization between European landraces, later exploiting more distant germplasm, particularly for disease resistances, e.g., *mlo-11* allele from an Ethiopian landrace which controls mildew resistance in most of the European spring barley elite varieties (Friedt and Rasmussen 2003). A remarkable achievement has been made in breeding winter barley varieties resistant to soil borne mosaic inducing viruses that causes significant yield losses in barley fields of the temperate world by utilizing the resistance resources present in the primary barley gene pool (Ordon et al. 2005).

In the recent years, breeding programs have been enhanced by the implementation of modern biotechnology tools, like the doubled haploid technique and marker-assisted selection procedures (Friedt and Rasmussen 2003). Highly efficient PCR-based DNA markers have been developed for some of the important disease resistance genes, e.g., *Rym4/Rym5* locus conferring resistance to barley yellow mosaic virus disease, *mlo11* for powdery mildew and *Rh2* for scald disease of barley (Ordon et al. 2004; Thomas 2003). These markers can be used to identify resistant genotypes independent of disease tests. Examples of the use of marker assisted selection (MAS) to improve quantitative traits have been reported in barley, e.g., for

stripe rust (Toojinda et al. 1998), Barley Yellow dwarf virus (Scheurer et al. 2001) and even yield (Schmierer et al. 2004).

Using the cytogenetic and molecular methods, agronomically useful recombinant lines containing introgressions from *H. bulbosum* have been developed, making it possible to extend the current working gene pool of barley beyond the primary gene pool (Pickering and Johnston 2005). Moreover, reliable methodologies are now available for the genetic transformation of barley using either direct DNA delivery by particle bombardment, or *Agrobacterium*-mediated gene delivery (Harwood et al. 2004). This enables efficient incorporation of genes of interest from diverse sources without changing the genetic background of the recipient cultivars.

During the last two decades the development of wide range of DNA markers (RFLP, RAPDs, AFLPs, SSRs, STSs and SNPs) and their use in genome analysis has provided unprecedented insight into structural features of the barley genome (Graner et al. 2004). There are over 40 published genome wide maps of barley. These maps are highly useful to localize economically important traits and to develop closely linked markers to these traits useful for marker assisted selection. A large set of barley ESTs (>430,000) is available in the public EST database of the NCBI (http://www.ncbi.nlm.nih.gov/dbEST/) which can be used as a resource for structural and functional analysis of the barley genome. Furthermore, high throughput whole genome profiling technique, i.e., Diversity Arrays Technology (DArT) has been developed for barley that can detect and type DNA variation at several hundred genomic loci in parallel without relying in sequence information (Wenzl et al. 2004).

The development of high yielding cultivars with improved quality and resistance/tolerance to biotic and abiotic stresses is the main aim of modern barley breeding. Among the several biotic factors that limit barley yield, fungal diseases, e.g., powdery mildew (*Blumeria graminis* f. sp. *hordei*) and leaf rust (*Puccinia hordei*), and yellow mosaic disease of barley caused by soil borne viruses, i.e., Barley mild mosaic virus (BaMMV) and Barley yellow mosaic virus (BaYMV) are of special importance because of the following reasons.

The fungal pathogens *B. graminis f. sp. hordei* and *P. hordei* are distributed worldwide; these pathogens are responsible for significant reduction in grain yield and its quality, and are characterized with wide spectra of pathogenic strains. Similarly, barley yellow mosaic inducing viruses are becoming a serious threat to the

winter barley crop in Europe and East Asia, because of constant spread of the viruses and evolution of new strains overcoming the resistance of elite winter barley cultivars. Therefore, emphasis has been given to these diseases in the present investigation in order to find out novel resistance sources, if there exist any within the Nepalese hulless barley germplasm.

1.6 Barley yellow mosaic disease

1.6.1 Disease status and resistance breeding

Barley yellow mosaic disease, caused by a complex of different strains of Barley yellow mosaic virus (BaYMV) and Barley mild mosaic virus (BaMMV), is one of the major constraints of winter barley cultivation in Europe and East Asia. The disease was first detected in Japan (Ikata and Kawai 1940) and later reported in Europe after about four decades (Huth and Lesemann 1978). The causal viruses belong to the genus *Bymovirus* within the family of Potyviridae and are characterized by a bipartite, single-stranded (+) sense RNA genome. The virus particles are transmitted into the root cells via the fungal vector *Polymyxa graminis* (Toyama and Kusaba 1970).

The virus infected plants show typical symptom of yellow or chlorotic streaks on leaves (1–5 mm in length) along the veins. Occasionally, the symptoms may appear on the leaf sheath as well. The symptoms are more distinct on young leaves and sometimes become necrotic, particularly towards the leaf tip. Up to 50 % yield losses may occur when susceptible barley cultivars are grown in severely infested soils. Due to the soil borne nature of the disease, i.e., viruses are transmitted by *P. graminis* which produces resting spores that can lie dormant but viable in soil for several decades and protect viruses from the environment for a long time and its presence up to a soil depth of 60 cm, chemical protection measures are neither effective nor acceptable for economical and ecological reasons. Furthermore, crop rotation is not adequate to eliminate the viruses from the soil. Therefore, the use of resistant cultivars is the most appropriate strategy to ensure successful barley cultivation in the infested fields.

In Europe, particularly in Germany, extensive studies have been carried out on barley yellow mosaic disease (Götz and Friedt 1993; Ordon and Friedt 1993), and a number of resistance genes have been identified and characterized (Ordon et al. 1993; Bauer et al. 1997; Werner et al. 2003a; Le Gouis et al. 2004). An overview on mapped resistance genes against barley yellow mosaic virus disease, the resistance of the

donor in Germany and the virus or virus strains used for mapping is given in Table 1 (Ordon 2005).

The goal of breeding high yielding barley cultivars with resistance to yellow mosaic inducing viruses was achieved in Europe quite rapidly in the last two decades (Friedt and Rasmussen 2003). The genetic basis of resistance has been mainly based on two recessive genes, *rym4* and *rym5* that are effective against the initially reported viral strains in Europe, i.e., BaMMV, BaYMV and BaYMV-2 (Huth 1989; Huth and Adams 1990). The gene '*rym4'* confers resistance to BaMMV and BaYMV but not BaYMV-2. Due to the increasing occurrence of BaYMV-2, *rym5* has become the gene of choice in European barley breeding which in addition to BaMMV and BaYMV, also confers resistance to BaYMV-2 (Friedt et al. 2000).

In contrast to the narrow genetic base of BaMMV/BaYMV resistance of European winter barley cultivars, the spectra of viral strains are widening. For example, new variants of BaMMV and BaYMV that overcome several resistance genes including *rym5* have been reported in France (Hariri et al. 2000; Hariri et al. 2003; Kanyuka et al. 2004). Likewise, a new BaMMV strain that overcomes *rym5* has also been detected in Germany (Ordon et al. 2005).

A more complex situation exists in East Asia from where at least seven strains of BaYMV and two of BaMMV are reported in Japan (Nomura et al. 1996), and a BaMMV strain that differs from the Japanese and German ones has been found in Korea (Lee et al. 1996). Similarly, several biological isolates of BaYMV have been recognized in China (Chen et al. 1996). The whole scenario reveals that there is a potential risk of resistance breakdown by new viral strains. Therefore, it is necessary to diversify the resistance genes within the winter barley breeding pool and to incorporate a broad spectrum durable resistance in elite winter barley cultivars (Werner et al. 2005).