Madhav Prasad Pandey

Molecular assessment of genetic diversity and population differentiation of hulless barley (*Hordeum vulgare* L.) landraces from the Himalayas of Nepal and its relevance for barley breeding

Cuvillier Verlag Göttingen

Aus dem Institut für Pflanzenbau und Pflanzenzüchtung I
der Justus-Liebig-Universität Gießen

Molecular assessment of genetic diversity and population differentiation of hulless barley (*Hordeum vulgare* L.) landraces from the Himalayas of Nepal and its relevance for barley breeding

Dissertation zur Erlangung des Doktorgrades (Dr. agr.) beim Fachberich Agrawissenschaften, Ökotrophologie und Umweltmanagement der Justus-Liebig-Universitat Gießen

vorgelegt von
Madhav Prasad Pandey
aus Nepal

Bibliografische Information Der Deutschen Bibliothek
Die Deutsche Bibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über http://dnb.ddb.de abrufbar.
1. Aufl. - Göttingen : Cuvillier, 2007
Zugl.: Gießen, Univ., Diss., 2006

ISBN: 978-3-86727-112-7

Gedruckt mit Unterstützung des Deutschen Akademischen Austauschdienstes (DAAD)

Tag der Disputation: 01.12.2006

Mitglieder der Prüfungskommission

Vorsitzender: Prof. Dr. Steffen Hoy

1. Gutachter: Prof. Dr. Dr. h.c. Wolfgang Friedt

2. Gutachter: Prof. Dr. Wolfgang Köhler

Prüfer: Prof. Dr. Bernd Honermeier

Prüfer: Prof. Dr. Andreas Vilcinskas

Nonnenstieg 8, 37075 Göttingen
Telefon: 0551-54724-0
Telefax: 0551-54724-21
www.cuvillier.de

1. Auflage, 2007
Gedruckt auf säurefreiem Papier

ISBN: 978-3-86727-112-7

Table of Contents

Acknowledgements

I am grateful to Prof. Dr. Dr. h.c. Wolfgang Friedt for excellent scientific advisory in his capacity as major supervisor, and for his encouragement, motivation and support to carry out this research project.

I am grateful to Prof. Dr. Wolfgang Köhler for his contributions in his capacity as my doctoral study supervisor.

I am highly indebted to Prof. Dr. Frank Ordon for his intensive guidance and motivation during the doctoral study, and for shaping up my skills to carry out this scientific investigation, successfully.

I especially thank Dr. Carola Wagner for her excellent expertise and support to carry out this research project, and also for the needful help in academic and administrative matters. Thanks are also due to my colleagues, Katrin Humbroich and Christof Stoll, and technical assistants Annette Plank and Swetlana Renner, for their helps in conducting laboratory and field experiments. I want to express my gratitude to our greenhouse and field station crews; especially, Petra Kretschmer and Roland Kürschner in the greenhouse, not only for their contribution in taking care of greenhouse experiments but also for a homely working environment.

I acknowledge Ulla Riedmeier, Sabine Schomber and Ingeborg Scholz (IPZ secretariat) for their cooperation and support.

I want to thank all colleagues and members from the Institute for Crop Science and Plant Breeding I. In particular, I am grateful to Dr. Rod Snowdon, Dr. Christian Obermeier and Dr. Jutta Ahlemeyer for highly constructive scientific discussions. Also, I am indebted to my colleague Hubert Adoukonou Sagbadja for a nice and friendly companion.

The financial support provided by the German Academic Exchange Service (DAAD) and barley samples provided by the Barley Germplasm Centre, Okayama University, Kurashiki, Japan is duly acknowledged.

Lastly, perhaps the most important to me is the patience, love and support from my family during my stay in Germany. My wife Manju, my daughter Mahima and my son Mahan deserve my sincere thanks.

List of Figures

List of Tables

Abbreviations

AFLP	amplified fragment length polymorphism
bp	base pair
CIA	Chloroform-isoamylalcohol
cm	centimeter
Cont.	continuation
cpSSR	chloroplast-SSR
CTAB	Cetyltrimethyl-ammoniumbromid
cvs	cultivars
DArT	Diversity Arrays Technology
DI	diversity index
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleicacid
dNTP	deoxyribonucleotide
EDTA	Ethylene di-amine tetra-acetate
EST	expressed sequence tag
EtOH	Ethanol
F1	1st filial generation
F2	2nd filial generation
FAO	Food and Agriculture Organization of the United Nations
GC	German cultivars
GS	genetic similarity
H_2O_{dd}	double distilled water
IAM	infinite allele model
IFZ	Interdisziplinäres Forschungszentrum
IPZ	Institut für Pflanzenbau und Pflanzenzüchtung I
K_2HPO_4	Potassium phosphate
LD	linkage disequilibrium
mA	milli-Ampere
MAS	marker assisted selection
MB	model based
$MgCl_2$	Magnesiumchloride
min	minute

mm	milli-meter
mM	milli-mole
NaOAc	Sodium acetate
NCBI	National Center for Biotechnology Information
ng	nano-gram
NH_4Oac	Ammonium acetate
NL	Nepalese landrace
nm	nano-meter
PCR	polymerase-chain-reaction
pg	pico-gram
pH	Hydrogen proton
PIC	polymorphism information content
QTL	quantitative trait locus
RAPD	random amplified polymorphic DNA
rDNA	ribosomal DNA
RFLP	restriction fragment length polymorphism
RNAse	ribonuclease
rpm	revolution per minute
SAHN	Sequential Agglomerative Hierarchical and Nested
SIMQUAL	similarity for qualitative data
SMM	stepwise mutation model
SNPs	single nucleotide polymorphisms
SSR	simple sequence repeats
TBE	Tris/Borate/EDTA-buffer
TEMED	Tetremethylethylenediamide
Tris	Tris-(hydroxymethyl)-aminomethan
UPGMA	Unweighted Pair Group Method with Arithmetic mean
vs	versus
µl	micro-liter

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).

Table 1 Mapped resistance genes against the barley yellow mosaic virus disease, their source, resistance of the donor in Germany, and virus used for mapping (Ordon et al. 2005)

Resistance genes	Chromo-some	Source	Resistance donor in Germany	Virus used for mapping	Reference
rym1	4HL	Mokusekko 3	BaMMV, BaYMV, BaYMV- 2	BaYMV[a]	Takahashi et al. (1973), Götz and Friedt (1993), Ordon et al. (1993), Konishi et al. (1997)
rym2	7HL	Mihori Hadaka 3	BaMMV, BaYMV, BaYMV- 2	BaYMV[a]	Takahashi et al. (1973), Götz and Friedt (1993), Ordon et al. (1993)
rym3	5HS	Ea 52, Ishuku-Shirazu	BaYMV, BaYMV-2	BaYMV[a]	Götz and Friedt (1993), Ordon et al. (1993), Saeki et al. (1999)
rym4	3HL	Ragusa, Franka	BaMMV, BaYMV	BaMMV, BaYMV	Götz and Friedt (1993), Graner and Bauer (1993), Ordon et al. (1993, 1995), Pellio et al. (2005)
rym5	3HL	Mokusekko 3, Resistant Ym No. 1, W122/ 37.1	BaMMV, BaYMV, BaYMV- 2	BaMMV, BaYMV, BaYMV- 2, BaYMV[a]	Götz and Friedt (1993), Ordon et al. (1993), Graner et al. (1995, 1999a), Konishi et al. (1997), Pellio et al. (2005)
rym6	3HL	Prior, Amagi Nijo	Susceptible	BaYMV[a]	Iida and Konishi (1994), Iida et al. (1999), Konishi et al. (2002)
rym7	1HS	HH or 3365	BaMMV	BaMMV	Graner et al. (1999b)
rym8	4HL	10247	BaMMV, BaYMV	BaMMV	Götz and Friedt (1993), Ordon et al. (1993), Bauer et al. (1997), Graner et al. (1999b)
rym9	4HL	Bulgarian 347	BaMMV	BaMMV	Götz and Friedt (1993), Ordon et al. (1993), Bauer et al. (1997)
rym10	3HL	Hiberna	BaYMV, BaYMV-2	BaYMV, BaYMV- 2	Graner et al. (1995, 1999a)
rym11	4HL	Russia 57	BaMMV, BaYMV, BaYMV- 2	BaMMV	Götz and Friedt (1993), Ordon et al. (1993), Bauer et al. (1997), Nissan- Azzouz et al. (2005)
rym12	4HL	Muju covered 2	BaMMV, BaYMV, BaYMV- 2	BaMMV	Götz and Friedt (1993), Ordon et al. (1993)
rym13	4HL	Taihoku A	BaMMV, BaYMV, BaYMV- 2	BaMMV	Götz and Friedt (1993), Ordon et al. (1993), Werner et al. (2003b)
Rym14 ^{Hb}	6HS	*Hordeum bulbosum*	BaMMV, BaYMV, BaYMV- 2	BaMMV, BaYMV, BaYMV- 2	Ruge et al. (2003)
rym15	6HS	Chikurin Ibaraki 1	BaMMV, BAYMV, BaYMV- 2	BaMMV	Le Gouis et al. (2004)
	5HS	Chikurin Ibaraki 1	BaMMV, BaYMV, BaYMV- 2	BaYMV, BaYMV- 2	Werner et al. (2003a)
Rym16 ^{Hb}	2HL	*Hordeum bulbosum*	BaMMV, BaYMV, BaYMV- 2	BaMMV, BaYMV, BaYMV- 2	Ruge et al. (2004)

a, Japanese strain of BaYMV

1.6.2 Genetics of BaMMV/BaYMV resistance

Due to the fact that barley yellow mosaic disease is caused by several viruses or viral strains (BaMMV, BaYMV, BaYMV-2), barley genotypes show a complex pattern of resistance reactions depending on the resistance genes being inherited. The resistance can be complete or partial, and against all the viruses or selective to some of them (Götz and Friedt 1993; McGrann and Adams 2004). Therefore, genetic analysis of resistance to BaMMV/BaYMV is difficult, which is further complicated due to varying infection rates in the field, particularly, in the case of BaYMV for which an artificial infection procedure is lacking. Within the germplasm of the primary gene pool, resistance is mainly conferred by recessive resistance genes (Götz and Friedt 1993; Ordon and Friedt 1993; Konishi et al. 1997). However, dominant genes have been reported in *H. bulbosum* (Ruge et al. 2003; Ruge et al. 2004). A number of resistance genes have been mapped on respective barley chromosomes (Table 1) and high resolution genetic maps have been developed for some of the important genes, e.g., *rym4/rym5*, *rym11* (Pellio et al. 2005; Nissan-Azzouz et al. 2005). More recently, the resistance locus *Rym4/Rym5* has been isolated and it has been shown that these are the allelic forms of the same gene, i.e., the eukaryotic translation initiation factor *eIF4E* (Stein et al. 2005; Kanyuka et al. 2005).

1.7 Nepalese Hulless barley genetic resource

Globally, over 280,000 accessions of barley genetic resources are conserved in numerous *ex situ* collections (Valkoun and Konopka 2004). Out of these, approximately 40% are landraces collected from different parts of the world. In this respect, barley landraces from the Himalayas, particularly from the highlands of Nepal, share a significant part of the world barley germplasm resources (Table 2). Due to the fact that naked barley is widely grown in the highlands of Nepal from the East to the West (about 800 km) along the Himalayas, it is frequently represented in Himalayan barley collections and can be considered as an important genetic resource.

The diversity of Himalayan barley is described by various authors. For example, Witcombe and Murphy (1986) assessed morphological variation, and Konishi and Matsuura (1991) analyzed isozyme genotypes of the Himalayan barley landraces and found hulless types highly differentiated from hulled ones. Based on isozyme diversity (Liu et al. 1999) and sequence variation at a DNA marker closely linked to

the *nud* locus (Taketa et al. 2004) hulless barley landraces from the highlands of Nepal turned out to be distinct from the Chinese, Korean and Japanese types.

Table 2 Most frequent origin of barley landraces in the international barley gene banks (Valkoun and Konopka 2004)

Origin	Numbers of accessions
Ethiopia	15,353
China	5,966
Turkey	5,884
Nepal	3,162
Switzerland	2,964
India	2,629
Pakistan	2,575
Russia	2,387
Afghanistan	1,509
Iran	1,509
Ukraine	1,275
Morocco	1,263
Total	46,549

In an extensive survey on Nepalese naked barley germplasm, Sharma et al. (1994) found a vast variation in morphology between and within landrace populations. The varying responses of Nepalese hulled and hulless barley landraces to diseases and agronomic performance are also reported by Baniya et al. (1997). Although information is sketchy, it hints to a high level of genetic diversity of Nepalese hulless barley landraces. However, despite a wider perspective for the exploitation of Himalayan barley genetic resource and in particular hulless barley landraces from the highlands of Nepal, detailed information on genetic diversity and population differentiation is lacking which is vital for the effective utilization and management of the genetic resources.

1.8 Molecular assessment of barley diversity and differentiation

Molecular methods have become indispensable tools in genetic diversity and population differentiation studies due to the practical importance of the information being generated (Rao and Hodgkin 2002). There are large number of literatures on genetic diversity and population analysis of barley using molecular markers. Some of the salient papers published in the past 10 years are summarized in the following paragraphs.

Ordon et al. (1997) analyzed the genetic relatedness of German/exotic barley cultivars and wild accessions (*H. spontaneum*) resistant to soil-borne mosaic-inducing viruses (BaMMV, BaYMV and BaYMV-2) using RAPDs, and reported that the UPGMA clustering based on genetic similarity precisely differentiated the barley accessions according to their known pedigree or origin. Furthermore, a considerable diversity in BaMMV/BaYMV-resistance genes was found within the exotic germplasm which can be highly useful in resistance breeding.

Russell et al. (1997) compared the level of polymorphism detected by RFLP, AFLP, SSRs and RAPDs markers analyzed on 18 barley cultivars. The SSRs detected the highest level of polymorphism compared to other three assays. Similarly, Struss and Plieske (1998) analyzed 163 barley accessions selected from the IPK Genebank (Gatersleben, Germany) that comprised landraces, cultivars and wild barley accessions (*H. spontaneum*; *H. agriocrithon*), with 15 SSRs, and reported a very high level of genetic diversity (Diversity index, DI=0.74). Moreover, the genetic diversity estimations separately on the three samples, i.e., 46 wild accessions, 52 landraces and 65 old cultivars and landraces, revealed that the wild barley possess the highest diversity (DI=0.75), however, the DI within the landraces and cultivars was also high (DI=0.72 and 0.70, respectively).

The isozyme analysis of East Asian accessions of the barley core collection revealed highly diverse Indian cultivars followed by Korean and Chinese ones (Liu et al. 1999). In this study, landraces from Bhutan and Nepal were found to be least polymorphic; however, Nepalese landraces represented some of the rare alleles, e.g., *Pgd-1* and *Tj*. Strelchenko et al. (1999) studied the genetic differentiation of barley from principal cultivation regions of the world using RAPDs, and reported three distinct groups that can be related to the evolutionary directions and geographical distribution of the crop. The first group indicated the westward distribution of barley from West Asia to Europe and New world across Ethiopia and then Mediterranean region. The second group was associated with eastward distribution of the crop and comprised the East Asian and central Asian accessions, and the third group represented the evolution and dissemination of hulless barley in central Asia and Caucasus region.

Ramsay et al. (2000) published a large set of barley SSRs (n=568), out of which 242 were mapped SSRs covering the 7 barley chromosomes. Soon after, Macaulay et al. (2001) developed a subset of 48 SSRs characterized by single locus robust alleles,

which are highly informative and cover the whole barley genome. These SSRs are highly useful for genotyping of barley in genetic studies, diversity estimation and population analysis.

Russell et al. (2000) analyzed 28 mapped barley SSRs on a large set of leading spring barley cultivars grown in northern Europe over the past 100 years, and found a reduction in diversity of modern cultivars compared to their key progenitors (DI=0.484 and DI=0.597, respectively). In contrast to this, Koebner et al. (2003) analyzed a large set of UK barley varieties released over the period 1925−1995 using phenotypic and genotypic (AFLP and SSRs) data and demonstrated a consistent pattern of diversity over the time. The authors concluded that systematic plant breeding does not inevitably lead to a reduction in the genetic diversity of agricultural crops.

Backes et al. (2003) studied the diversity patterns in major groups of barley cultivars and landraces of Europe using RFLP markers, and found a comparable diversity between the landraces and improved cultivars. However, the genetic diversity detected in winter barley was significantly higher than that of spring type (diversity estimates, H=0.415 and H=0.260, respectively). Furthermore, Russell et al. (2003) studied the genetic differentiation of barley landraces sampled from five different ecogeographical regions of Syria and Jordan, with nuclear and chloroplast SSRs (cpSSRs), and reported a difference in the patterns of population differentiation detected by these markers. A high level of population differentiation was detected within and between the sites with cpSSRs (F_{ST} =0.45 and F_{ST} =0.44), whereas, nuclear SSRs revealed most of the genetic variation within the sites. A significant differentiation between the sites was also detected with nuclear SSRs (F_{ST} =0.29), however, the F_{ST} −*value* was less than that estimated with the cpSSRs.

Hamza et al. (2004) assessed the genetic diversity of 26 Tunisian winter barley cultivars/landraces with 17 SSRs, and reported a moderate level of diversity in this sample (DI=0.45). Moreover, the UPGMA cluster analysis based on SSRs data and morphological data clearly differentiated the local landraces and modern varieties. A good correspondence was found between the clusters based on SSRs and morphological data.

In a retrospective analysis of genetic diversity in winter barley, Ordon et al. (2005) found a different pattern of allele distribution between two and six-rowed cultivars and

changes of allele frequencies in relation to the time of release. Furthermore, it was found that the diversity of two-rowed cultivars increased over time compared to the older cultivars. Chabane et al. (2005) compared the diversity detected by the genomic and EST−derived SSRs in wild (*H. spontaneum*) and cultivated barley, and found a higher polymorphism information content of genomic SSRs than that of EST−SSRs. However, EST−SSRs indicated a clearer separation between the wild and cultivated barley. The EST−SSRs represent the expressed genomic regions and therefore, provide a direct estimation of functional biodiversity (Chabane et al. 2005).

Malysheva-Otto et al. (2006) analyzed a large number of cultivated barley accessions (n=953) originating from all inhabited continents, except Australia, with 48 SSRs. The diversity estimations within different groups revealed a lower level of diversity in European accessions (average gene diversity=0.64) than those derived from Near East (0.78) and Asia (0.74). Furthermore, the global population of cultivated barley was found to be highly structured. Linkage disequilibrium (LD) in this sample extended up to 50 cM, and was strongly dependent on the population structure.

In recent time, SSR markers are widely used for diversity assessment in different crop species, e.g., rice (Jain et al. 2004), maize (Patto et al. 2004), wheat (Khlestkina et al. 2004) and sorghum (Uptmoor et al. 2003; Abu-Assar et al. 2005). In barley large numbers of barley SSRs are available, of which, the SSRs set proposed by Macaulay et al. (2001) for genotyping in barley is highly informative and gains equispaced genome coverage. In the present investigation, a large set of hulless barley landraces originally collected from the highland of Nepal (Himalayas) was analyzed with a panel of 44 mapped SSRs in order to get a precise estimate of genetic diversity and an in depth view on population structure. Moreover, to provide a genetic basis for the utilization of Nepalese hulless barley genetic resources in mainstream barley breeding, the genetic relatedness between the Nepalese hulless barley and mainstream barley cultivars derived from East Asia, Europe and North America was analyzed. Furthermore, the landraces were evaluated for reaction to virus (BaMMV) and fungal pathogens (*B. graminis* f. sp. *hordei*; *P. hordei*).

2. Materials and Methods

2.1 Genetic diversity and population differentiation analysis of Nepalese hulless barley landraces

2.1.1 The hulless barley samples and geographic origins

In total 107 hulless barley accessions were included in this study, comprising 106 landraces derived from the gene bank of the Barley Germplasm Center, Okayama University, Kurashiki, Japan and the cultivar 'Solu-Uwa' released for the high mountain regions in Nepal (Table 3). The hulless barley samples analyzed are six-rowed spring types; however, some are of intermediate growth habit. The detailed descriptions on growth habit, morphology and important agronomic traits are available in the Catalogue of Barley Germplasm Preserved in Okayama University, 1983.

The barley landraces were originally collected from the Annapurna and Manaslu-Himalaya-Range covering the six administrative districts of central Nepal (Mustang, Manang, Myagdi, Kaski, Lamjung and Gorkha) (Figure 3).

Figure 3 Map of Nepal showing the landrace collection sites indicated with shaded areas

The geographic location of the study area is between 28°15' and 28°55' in the North, and 83°35' and 85°05' in the East. This includes the areas both on the South as well as North slopes of the main Himalayas-crest. The barley samples used originated from the wider parts of the famous Annapurna and Manaslu conservation areas and

represent the highlands, valleys and mountain terraces in the catchments of rivers BudhiGandaki, Marshyangdi and KaliGandaki in central Nepal. The altitude in this region varies greatly from the floor of the valleys (500-4000 m) to the top of the Himalayas (>8000 m). Similarly, a strong South-to-North monsoon gradient causes a wide difference in annual precipitation in the region ranging from precipitation peaks at 5032 mm yr^{-1} at about 3000 (m) altitude on the southern side to ~1100 mm yr^{-1} in the rain shadow to the North of the main Himalayas-crest (Putkonen 2004). Because of the vast topographical heterogeneity and impact of the Himalayas on amount and distribution of precipitation, the barley samples used in the present study represent highly diverse and isolated eco-geographic locations.

Using a high resolution (1:25000) topographical map of the study area (Survey Department, Govt. of Nepal), and information provided on collection sites (Catalogue of Barley Germplasm Preserved in Okayama University 1983) or the landraces' names which in general correspond to the locality of collection, a combined map of the entire region was developed and origins of landraces were located (Figure 4).

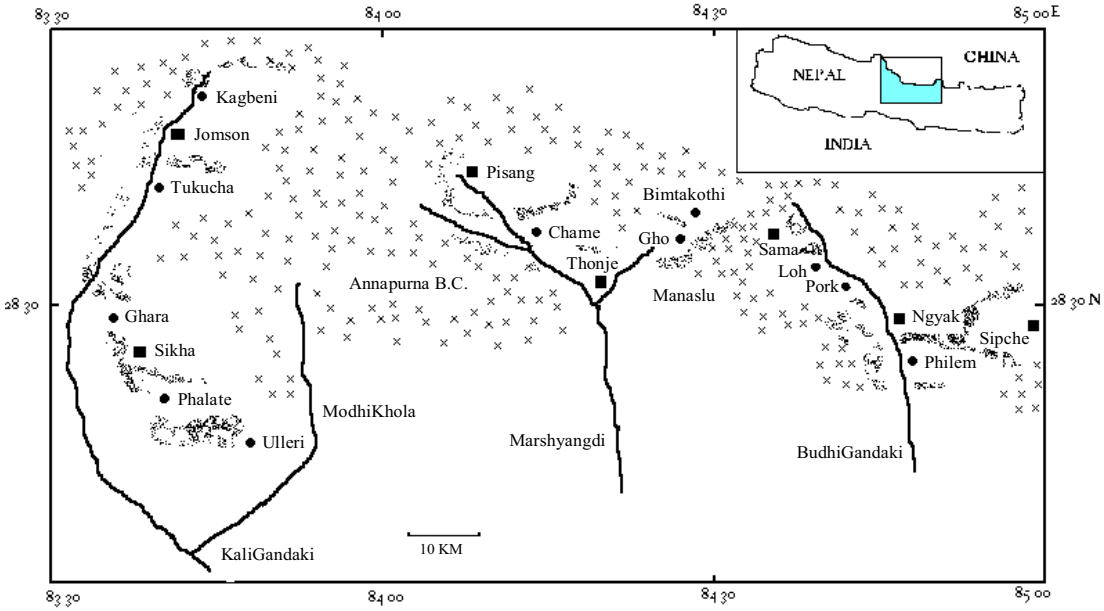

Figure 4 Map of the upper basins of rivers KaliGandaki, Marshyandi and BudhiGandaki extended along the Annapurna and Manaslu Himalaya-range in central Nepal. The positions are drawn with an approximate scale. X indicates permanently snow covered mountains and shaded patches are indicative for dense pine or mixed forest. The locations marked with a shaded square comprise distinct barley populations

Table 3 Origin, geographic grouping and sample size of the hulless barley landraces analyzed

Geographic region	Origin	Nos. of landraces	Landraces
Upper basin of KaliGandaki	Jomson	2	Jomson-1, Jomson-2
	Kagbeni	2	Kagbeni-3, Kagbeni-5
	Tukucha	1	Tukucha
	Dhumpu	1	Dhumpu-2
	Total	6	
Lower basin of KaliGandaki	Sikha	7	Sikha-1, Sikha-2, Sikha-4, Sikha-5, Sikha-6, Sikha-7, Sikha-8
	Ulleri	2	Ulleri-9, Ulleri-21
	Ghara	2	Ghara-1, Ghara-2
	Phalatey	1	Phalatey
	Total	12	
	Annapurna-BC	2	Annapurna BC-1, Annapurna BC-2
Upper basin of Marshyangdi	Chame	8	Chame-2, Chame-3, Chame-8, Chame-9, Chame-11, Chame-12, Chame-13, Chame-14
	Pisang	6	Pisang-4, Pisang-5, Pisang-6, Pisang-7, Pisang-8, Pisang-9
	Thonje	8	Thonje-3, Thonje-4, Thonje-5, Thonje-6, Thonje-16, Thonje-18, Thonje-19, Thonje-21
	Gho	3	Gho-1, Gho-2, Gho-3
	Total	25	
	Bimtakothi	10	Bimtakothi-1, Bimtakothi-2, Bimtakothi-3, Bimtakothi-4, Bimtakothi-5, Bimtakothi-9, Bimtakothi-10, Bimtakothi-11, Bimtakothi-12, Bimtakothi-13
Upper basin of BudhiGandaki	Ngyak	7	Ngyak-1, Ngyak-2, Ngyak-3, Ngyak-4, Ngyak-10, Ngyak-11, Ngyak-12
	Sama	7	Sama-1, Sama-2, Sama-3, Sama-4, Sama-6, Sama-8, Sama-9
	Philem	3	Philem-1, Philem-2, Philem-3
	Pork	2	Pork-1, Pork-2
	Total	19	
East of BudhiGandaki	Sipche	8	Sipche-2, Sipche-3, Sipche-4, Sipche-6, Sipche-7, Sipche-9 Sipche-11, Sipche-12
	Total	8	
	Thomje	5	Thomje-2, Thomje-3, Thomje-4, Thomje-6, Thomje-7
	Thangja	3	Thangja-1, Thangja-2, Thangja-3
	Tilman Camp	3	Tilman Camp-1, Tilman Camp-7, Tilman Camp-8
	Lih Dharna Gal	1	Lih Dharna Gal
	Tsumje	2	Tsumje-1, Tsumje-2
	(Unknown)	11	Naked-304, N-6, N-12, Solu Uwa, Nepal-1, Nepal-2, Nepal-3, Nepal-4, Nepal-5, Nepal-6, Nepal-7
Total		107	

The landrace origins were divided into five geographic regions following the three river systems: (1) upper basin of KaliGandaki, (2) lower basin of KaliGandaki, (3) upper basin of Marshyangdi, (4) upper basin of BudhiGandaki, and (5) East of BudhiGandaki (Table 3). The accessions derived from Bimtakothi, Annapurna BC, Thomje, Thangja, Tilman camp and Tsumje are not included within the five geographic groups and considered as independent groups according to their origin. Of these, Bimtakothi and Annapurna BC are relatively isolated locations, whereas the positions of Thomje, Thangja, Tilman camp and Tsumje are not indicated in the map because of ambiguity due to the differences in landrace names and the corresponding locality given in the topographic map. The origin of 11 landraces was not known. The seeds of 106 hulless barley landraces were obtained from the Barley Germplasm Center, Okayama University, Kurashiki (Japan) and multiplied in a greenhouse at the Institute for Crop Science and Plant Breeding I (Giessen) during the winter 2002/03 ensuring self pollination. The self-pollinated heads of each accession were harvested in bulk, and the seeds were used for molecular analysis and field experiments.

2.1.2 Molecular genotyping

2.1.2.1 DNA extraction

The genomic DNA was extracted from the bulk leaf tissues of five plants of each accession grown for about two weeks in 33 x 51.5 cm Quick pot standard plates in the greenhouse, following the CTAB DNA extraction protocol (DOYLE and DOYLE 1990). A leaf sample of 100−200 mg was crushed into fine powder using 200-250 ml liquid nitrogen and transferred into a pre-chilled 1.5 ml Eppendorf tube. Then, 700 µl extraction buffer (Table 4) was added and mixed thoroughly using a vortex. The sample was incubated for 20-30 minutes at 65°C in the water bath and 700 µl 24:1 chloroform-isoamylalcohol (CIA) was added, mixed gently by shaking for about 5 minutes and centrifuged at 10,000 rpm for 10 minutes. The liquid phase was transferred into a new 1.5 ml Eppendorf tube and filled with 600 µl CIA, mixed and centrifuged at 10,000 rpm for 10 minutes. The top liquid phase was transferred into a new 1.5 ml Eppendorf tube, 50 µl NH_4OAc (10 M) and 60 µl NaOAc (3 M, pH 5.5) was added and mixed gently. After that, 500µl 2-Propanol (4°C) was added and mixed gently by shaking until DNA precipitation occurred. A centrifugation step of 4,000 rpm for 4 minutes settled the solid phase. The liquid phase was then removed

and the DNA was washed with 200 µl EtOH (70 %)–NH_4OAc (10 mM) for about 10 minutes. After removing the alcohol and drying, the DNA was dissolved in 100 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) and 1 µl RNAse was added to digest RNA. The DNA concentration was measured with a Hoefer TKO fluorometer using H33258 dye (Hoechst) emitting light of 460 nm.

Table 4 DNA extraction buffer (Doyle and Doyle 1990)

NaCl	1.4 M
Tris HCl (pH 8.0)	0.1 M
EDTA (pH 8.0)	20 mM
CTAB	2 %
$Na_2S_2O_5$	1%
Mercaptoethanol	0.2 %

2.1.2.2 SSR assays

Forty-four simple sequence repeats (SSRs) markers were analyzed. Of this, 40 correspond to the SSRs set proposed by Macaulay et al. (2001) for genotyping in barley, and SSRs Bmac0154, Bmac0029, Bmag0385 and Bmag0007 were additionally selected for the present study. The repeat motifs and primer sequences of the SSRs are presented in Table 5. The PCR assay was carried out according to Ramsay et al. (2000) with modifications in a Geneamp 9700 thermal cycler (Perkin-Elmer).

The PCR protocols used for each of the 44 SSRs are given in Table 6. The final 20 µl PCR reaction volume consisted of 2 µl DNA probe (25ng/µl), defined amounts of PCR buffer, dNTPs, $MgCl_2$, forward (F) and reverse (R) primers, Taq-DNA polymerase, and H_2O_{dd} to make the final volume (Table 7). The details of thermocycler programs used are given in Table 8. The PCR products were resolved with polyacrylamide gel using an automatic DNA analyzer, Li-COR 4200 (MWG Biotech AG, Ebersberg). The compounds for a 0.25 mm polyacrylamide gel (Plate size: 25 X 25.5 cm) are 25 ml long ranger solution (16 ml 50 % FMC long ranger polyacrylamide, 42 g Urea (USB, Cleveland), 10 ml 10X TBE and H_2O_{dd} ad 100 ml), 25 µl TEMED (Sigma, Steinheim), 250 µl DMSO (Sigma, Steinheim) and 175 µl APS (Roth, Karlsruhe).

Table 5 Forty-four SSRs used in the study, primer sequences and repeat motifs (Ramsay et al. 2000; Macaulay et al. 2001)

SSRs	Primer sequence	Repeat motifs
Bmac0399	F- CGATGCTTTACTATGAGAGGT R- GGGTCTGAAGCCTGAAC	(AC)21
Bmac0154[a]	F- CTGGGTGATGAATAGAGTTTC R- TATTCTTCAAAAGATGTTCTGC	(AT)19(AC)6
Bmac0032	F- CCATCAAAGTCCGGCTAG R- GTCGGGCCTCATACTGAC	(AC)7T(CA)15(AT)9
Bmag0211	F- ATTCATCGATCTTGTATTAGTCC R- ATTCATCGATCTTGTATTAGTCC	(CT)16
HvHVA1	F- CATGGGAGGGGACAACAC R- CGACCAAACACGACTAAAGGA	(ACC)5
WMC1E8	F- TCATTCGTTGCAGATACACCAC R- TCAATGCCCTTGTTTCTGACCT	(AC)24
Bmac0093	F- CGTTTGGGACGTATCAAT R- GGGAGTCTTGAGCCTACTG	(AC)24
Bmac0134	F- CCAACTGAGTCGATCTCG R- CTTCGTTGCTTCTCTACCTT	(AC)28
Bmag0378	F- CTTTTGTTTCCGTAGCATCTA R- ATCCAACTATAGTAGCAAAGCC	(AG)14
EBmac0415	F- GAAACCCATCATAGCAGC R- AAACAGCAGCAAGAGGAG	(AC)17
HVM36	F- TCCAGCCGACAATTTCTTG R- AGTACTCCGACACCACGTCC	(GA)13
HVM54	F- AACCCAGTAACACCTGTCCTG R- AGTTCCCTGACCCGATGTC	(GA)14
Bmac0029[a, b]		
Bmac0067	F- AACGTACGAGCTCTTTTTCTA R- ATGCCAACTGCTTGTTTAG	(AC)18
Bmac0209	F- CTAGCAACTTCCCAACCGAC R- ATGCCTGTGTGTGGACCAT	(AC)13
Bmag0013	F- AAGGGGAATCAAAATGGGAG R- TCGAATAGGTCTCCGAAGAAA	(CT)21
Bmag0136	F- GTACGCTTTCAAACCTGG R- GTAGGAGGAAGAATAAGGAGG	(AG)6-(AG)10-(AG)6
Bmag0225	F- AACACACCAAAAATATTACATCA R- CGAGTAGTTCCCATGTGAC	(AG)26
HVM62	F- TCGCGACCAGACGAGAAG R- AGCTAGCCGACGACGCAC	(GA)11
Bmag0353	F- ACTAGTACCCACTATGCACGA R- ACGTTCATTAAAATCACAACTG	(AG)21
Bmag0384	F- TGTGAGTAGTTCACCATAGACC R- TGCCATTATCATTGTATTGAA	(AG)18
EBmac0701	F- ATGATGAGAACTCTTCACCC R- TGGCACTAAAGCAAAAGAC	(AC)23
HVM40	F- CGATTCCCCTTTTCCCAC R- ATTCTCCGCCGTCCACTC	(GA)6(GT)4(GA)7
HVM67	F- GTCGGGCTCCATTGCTCT R- CCGGTACCCAGTGACGAC	(GA)11
HvMLO3	F- CTTCCATGTCACCTACAG R- CGAACTGGTATTCCAAGG	(CTT)6
Bmac0113	F- TCAAAAGCCGGTCTAATGCT R- GTGCAAAGAAAATGCACAGATAG	(AT)7(AC)18
Bmag0222	F- ATGCTACTCTGGAGTGGAGTA R- GACCTTCAACTTTGCCTTATA	(AC)9(AG)17

Table 5 Cont.

SSR	Primer sequence	Repeat motifs
Bmag0223	F- TTAGTCACCCTCAACGGT R- CCCCTAACTGCTGTGATG	(AG)16
EBmac0684	F- TTCCGTTGAGCTTTCATACAC R- ATTGAATCCCAACAGACACAA	(TA)7(TG)11- (TG)11(TTTG)5
EBmac0970	F- ACATGTGATACCAAGGCAC R- TGCATAGATGATGTGCTTG	(AC)8
HvLOX	F- CAGCATATCCATCTGATCTG R- CACCCTTATTTATTGCCTTAA	(AG)9
HVLEU	F- TTGGAAGTGTACAGCAATGGAG R- TGAAAGGCCCCACAAGATAG	(ATTT)4
Bmac0018	F- GTCCTTTACGCATGAACCGT R- ACATACGCCAGACTCGTGTG	(AC)11
Bmac0040	F- AGCCCGATCAGATTTACG R- TTCTCCCTTTGGTCCTTG	(AC)20
Bmac0316	F- ATGGTAGAGGTCCCAACTG R- ATCACTGCTGTGCCTAGC	(AC)19
Bmag0009	F- AAGTGAAGCAAGCAAACAAACA R- ATCCTTCCATATTTTGATTAGGCA	(AG)13
Bmag0218	F- CATAGAGAGGGAGGGAGAG R- TCAACCTTACTGCATCTTTG	(AG)6(AG)6
EBmac0806	F- ACTAAGTCCTTTCACGAGGA R- GTGTGTAGTAGGTGGGTACTTG	(AC)4(GA)(CA)8-(CA)5
Bmac0273	F- ACAAAGCTCGTGGTACGT R- AGGGAGTATTTCACCCTTG	(AC)20(AG)20
Bmag0385[a]	F-CTCCACAGAGTCAGAGTTAGA R-CTGACATTAGCTGACTCTCTATC	(AG)18(TG)10
Bmag0007[a]	F-TGAAGGAAGAATAAACAACCAACA R-TCCCCTATTATAGTGACGGTGTG	(AG)16(AC)16
Bmag0120	F- ATTTCATCCCAAAGGAGAC R- GTCACATAGACAGTTGTCTTCC	(AG)15
Bmac0156	F- AACCGAATGTATTCCTCTGTA R- GCCAAACAACTATCGTGTAC	(AC)22(AT)5
HVCMA	F- GCCTCGGTTTGGACATATAAAG R- GTAAAGCAAATGTTGAGCAACG	(AT)9

a, Not included in the set of SSRs proposed by Macaulay et al. (2001)

b, Further information is confidential and subject to commercial license (Scottish Crop Research Institute, Invergowire, Dundee DD2 5DA, Scotland, UK)

Shortly before loading, 20 μl fuchsine buffer (95 ml formamide, 2 ml EDTA, 0.1 g basic fuchsine, 0.01g brome-phenol-blue, all reagents Sigma Steinheim, and H_2O_{dd} ad 100 ml) was added in the sample and denatured at 94º C for 1 minute and 30 seconds. A volume of 0.7 μl sample was loaded in the gel and electrophoresis was performed using 1X TBE buffer (1340 mM tris HCl, 450 mM boric acid, 25 mM EDTA, Sigma Steinheim, H_2O_{dd} ad 1l) and LiCOR setting for power supply at 1500 V, 50 W, 35 mA and plate temperature at 48º C. The SSR alleles were automatically detected by a dual laser system of the LiCOR based on 5' end label of the forward primer (IRD 700 or 800). The molecular weight of a SSR allele was estimated using a 50-700 (bp)

ladder (MWG Biotech, Ebersberg). The details of sample plan for gel electrophoresis are given in the section 2.2.2.

Table 6 PCR mixture and cycler program used for the 44 SSRs

SSRs	IRD	Cycler program	Recipe	SSRs	IRD	Cycler program	Recipe
Bmac0399	700	D	2	HVM40	800	A	7
Bmac0154	700	E	2	HVM67	700	A	5
Bmag0032	700	D	4	HvMLO3	700	D	1
Bmag0211	700	F	6	Bmac0113	800	F	2
HvHVA1	700	E	2	Bmag0222	800	F	8
WMC1E8	700	E	3	Bmag0223	800	F	2
Bmac0093	700	E	2	Ebmac0684	800	F	1
Bmac0134	700	E	6a	Ebmac0970	700	F	1
Bmag0378	700	F	2	HvLOX	800	F	9
Ebmac0415	700	D	2b	HVLEU	700	D	2
HVM36	800	A	2	Bmag0018	800	D	1
HVM54	700	A	2	Bmac0040	800	E	2a
Bmac0029	700	(Bmac29)	3	Bmac0316	700	E	1
Bmac0067	700	E	6	Bmag0009	800	F	1
Bmac0209	700	F	2	Bmag0218	800	F	2
Bmag0013	700	F	2	Ebmac0806	800	F	1
Bmag0136	800	F	2	Bmac0273	800	E	2
Bmag0225	700	F	2	Bmag0385	800	F	2
HVM62	700	A	2	Bmag0007	800	F	2
Bmag0353	800	F	2	Bmag0120	700	F	2
Bmag0384	800	F	2	Bmac0156	700	E	2
Ebmac0701	700	D	2	HVCMA	800	D	2

Table 7 Composition and amount (µl) of different PCR recipes used for the SSRs

PCR mixture\ Recipe	1	2	2a	2b	3	4	5	6	6a	7	8	9
DAN-probe (25 ng)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
H_2O_{dd}	6.7	10.7	10.7	10.7	13.2	10.5	12.7	13.1	13.1	13	10.3	11.9
Buffer (10X)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
dNTPs (10 mM)	0.4	0.4	0.4	0.4	0.2	0.6	0.4	0.4	0.4	0.4	0.4	0.4
$MgCl_2$ (25 mM)	0.8	0.8	0.8	0.8	0.4	0.8	0.8	0.4	0.4	0.5	1.2	1.6
Primer (Reverse)	4 (2)	2 (2)	2 (5)	2 (2)	1 (2)	2 (5)	1 (5)	1 (5)	1 (5)	1 (5)	2 (2)	1 (5)
Primer (Forward)	4 (2)	2 (2)	2 (5)	2 (1)	1 (2)	2 (5)	1 (5)	1 (5)	1 (2)	1 (5)	2 (2)	1 (5)
Taq (5U/µl)	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1

The figures in parenthesis indicate the primer concentration in pico-moles

(Taq-polymerase, $MgCl_2$, dNTPs, buffers; all from Eppendorf, Hamburg)

Table 8 Details of thermocycler programs used for the amplification of SSRs

Program	Initial denaturation	Polymerization	Final polymerization
A	94ºC for 3 min	(10 cycles) denaturation: 94º C for 1 min, annealing: 64º C (-1º C/ cycle) for 1 min, extension: 72º C for 1 min (30 cycles) denaturation: 94º C for 1 min, annealing: 55º C for 1 min, extension: 72º C for 1 min	72º C for 5 min
D	94ºC for 3 min	(1 cycle) annealing : 66º C for 1min, extension : 72º C for 1 min (5 cycles) denaturation : 94º C for 30 sec, annealing: 65º C (-1º C/ cycle) for 30 sec, extension: 72º C for 30 sec (24 cycles) denaturation: 94º C for 30 sec, annealing: 60º C for 30 sec, extension: 72º C for 30 sec	72º C for 5 min
E	94ºC for 3 min	(1 cycle) annealing: 55º C for 1 min, extension: 72º C for 1 min (30 cycles) denaturation: 94º C for 1 min, annealing: 55º C for 1 min, extension: 72º C for 1 min	72º C for 5 min
F	94ºC for 3 min	(1 cycle) annealing: 58º C for 1 min, extension: 72º C for 1 min (30 cycles) denaturation: 94ºC for 1 min, annealing: 58º C for 1 min, extension: 72º C for 1 min	72º C for 5 min
Bmac29	94ºC for 3 min	(45 cycles) annealing: 55º C for 30 sec, extension 72º C for 30 sec	72º C for 5 min

2.1.3 Statistical analysis

2.1.3.1 Estimation of genetic diversity and genetic relatedness

The alleles of each SSR were scored in molecular weight (bp) followed by transformation into binary codes as presence (1) or absence (0) of the allele using the software RFLP-scan 2.1 (Scanalytics). Double bands due to residual heterozygosity were not scored and considered as missing value in the statistical analysis. The polymorphism information content (PIC) of the SSRs was calculated according to Weber (1990):

$$PIC = 1 - \left(\sum_{i=1}^{n} p_i^2 \right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2\, p_i^2 p_j^2$$

where p_i and p_j are the frequencies of the i^{th} and j^{th} alleles in a given population. Using the 0/1 data matrix, genetic similarity between the genotypes was estimated

with DICE coefficient (Dice 1945) using the SIMQUAL method of the software package NTSYS-pc version 2.1 (Rohlf 2000) (Exeter Software, Setanket, NY). The DICE similarity coefficient is defined as: DICE= *2a/ (2a+b+c)*, where *a*=number of positive matches, and *b+c*=number of no matches. Based on DICE similarity matrix, UPGMA clustering of the landraces was carried out using the Sequential Agglomerative Hierarchical and Nested (SAHN) method of the software NTSYS-pc. The genetic diversity index (DI) was estimated as the mean gene diversity over the loci and adjusted for the sample size according to Nei (1978):

$$DI = 2n_a(1/n_l \sum_j (1 - \sum_i x_{ij}^2))/(2n_a - 1)$$

where x_{ij} is the frequency of the *i*th allele of locus *j*, n_l is the number of genetic loci, and n_a is the number of accessions.

2.1.3.2 Population structure analysis

The Bayesian model based approach proposed by Pritchard et al. (2000) was used to determine the population structure of the landraces. The model assumes *k* number of populations (where k may be unknown) characterized with a set of allele frequencies at each locus that are in Hardy-Weinberg equilibrium. The application tests for the presence of a population structure (*k*>1) and assigns the individuals from the sample population into groups for a given number of populations (*k*) in a way that Hardy-Weinberg disequilibrium and linkage disequilibrium (LD) are maximally explained. The software package STRUCTURE version 2.0 (Pritchard et al. 2000) was used to perform the analysis. The molecular weight data were used as an input file in haploid format similar to Kraakman et al. (2004). With the knowledge of UPGMA clusters analysis, STRUCTURE software was run for a presumed population number (*k*) from 1 to12, following the admixture ancestry model. Initially, a run length of 10,000-burn-in and 30,000 iterations after burn-in was performed. The run length was increased to 50,000-burn-in and 100,000 iterations after burn-in to achieve consistent results over repeated runs for each value of k, and to keep the alpha constant. The run with maximum likelihood was used to assign landraces to groups, and to reveal the group membership probability (inferred ancestry) of the landraces. Landraces with ≥90% inferred ancestry were considered to constitute a distinct population and those with <90 % were considered as admixtures.

2.1.3.3 Estimation of population diversity and differentiation

The populations identified with the structure analysis were characterized for genetic diversity and differentiation. The genetic variation within each population was described in terms of the number of polymorphic SSRs detected, mean number of alleles per locus, and thereafter by DI. Nei's unbiased genetic distance (Nei 1978) between populations was computed and the genetic relationship among the populations was revealed with the UPGMA cluster analysis. All the calculations described above were carried out using the software POPGENE version1.32 (Yeh et al. 1999).

Population differentiation was quantified with the parameters, θ (Weir and Cockerham 1984) which is analogous to F_{ST} (F-statistics, Wright 1951), and R_{ST} (R-statistics, Slatkin 1995). The θ is calculated on the variances of allele frequencies and defines the relatedness of pairs of alleles within a population relative to the total population. In contrast to this, R_{ST} is an estimator of the genetic differentiation based on the variance of allele size and is designed for genetic markers undergoing a strict stepwise mutation model (SMM). The detail definition of the two parameters is given in the following box.

The parameter R_{ST} (Slatkin 1995) is defined as:	The parameter θ (Weir and Cockerham 1984) is defined as:
$R_{ST} = (S - S_w) / S_w$	$\theta_w = \sum_l \sum_U a_{lu} \Big/ \sum_l \sum_u (a_{lu} + b_{lu} + c_{lu})$
where S is the average squared difference of allele size between all pairs of alleles, and S_w, is the average sum of squares of the differences of allele size within each sub-populations	where θ_w is the weighted average of θ estimated over u^{th} allele and j^{th} locus; *a, b* and *c* are the allele frequency variance components: *a*=between populations, *b*=between individuals within populations, *c*=between gametes within individuals

F_{ST} and R_{ST} are the most commonly reported parameters to describe population structure; however, they differ in sensitivity when estimated on SSRs (Balloux and Moulin 2002). The F_{ST} basically assumes the infinite allele model (IAM) and allelic equilibrium at loci thereby underestimating the magnitude of differentiation when

populations are highly structured or in a situation when SSR exhibit a high mutation rate. Contrarily, R_{ST} is independent of the mutation rate, however, suffers from high associated variance and any deviation from the assumed mutation model (SMM). For comparison and cautious interpretation of the results, both parameters were estimated (F_{ST} and R_{ST}). The software program FSTAT version 2.9.3.2 (Goudet 2002) was used to compute θ and R_{ST} (unbiased, Goodman 1997) without assuming random mating among the samples. A significance test of population differentiation (pairwise θ) and genotypic disequilibrium was performed by randomizing samples to obtain the log-likelihood G-statistics (Goudet et al. 1996). The significance tests were performed by conducting bootstrapping on loci with a 95% nominal confidence interval, and sequential Bonferroni correction was implemented for multiple tests (Rice 1989). The linear association between genetic differentiation (pairwise θ) and the geographic distance (hypothesis: isolation by distance) was tested by correlating the (θ/1- θ) matrix against the log-geographical distance matrix as suggested by Rousset (1997). The calculations and test of significance were performed according to the Mantel matrix correspondence test (Mantel 1967) using the software FSTAT version 2.9.3.2.

2.2 Genetic relationship of Nepalese hulless barley landraces with East Asian and Western barley cultivars

The analysis was performed by combining SSR data of the present experiment, i.e., on the set of Nepalese landraces, with the previously analyzed SSRs data on German and exotic barley cultivars at the Institute of Crop Science and Plant Breeding I, Giessen. The details on materials and methods used are presented in the following sections.

2.2.1 Plant material

In total 161 barley genotypes were analyzed. This includes 107 Nepalese hulless barley landraces studied for genetic diversity and differentiation (Table 3), five Canadian naked cultivars (Crop Development Center, University of Saskatchewan) and three German hulled cultivars (for these 115 genotypes SSR genotyping was performed in the present experiment), and a set of 46 hulled cultivars/accessions (all winter types) previously analyzed by Stoll et al. (2002) that comprised of 32 German cultivars released in between 1891 to 2000 AD, 12 exotic cultivars derived from East

Asia, East Europe and USA, and two *H. spontaneum* accessions derived from Israel (Table 9).

2.2.2 Alignment of SSR alleles between the experiments

In total 30 SSRs were used for the genetic analysis (Table 16). The SSRs alleles detected in the present experiment, i.e., on the set of 115 barley genotypes, were compared and aligned with those reported by Stoll et al. (2002). The strategy to bring the alleles of the two experiments on a common gel-platform is illustrated in Figure 5. For each of the 30 SSRs, the alleles reported by Stoll et al. (2002) were represented by corresponding genotypes used as internal standards in the present experiment. The gel-electrophoresis of 115 samples (present experiment) along with the internal standards (IS) was carried out in two separate gels (0.25 mm, plate size: 25 X 25.5 cm) using a 64-lane comb (Figure 5). In the case number of alleles in the IS ranged from 4 to 9, each of the three designated IS lanes accommodated more than one samples. However, for more than 9 alleles, a separate gel was used to align the alleles detected in the two experiments.

Gel-A (62 samples)

Size marker	Internal std.							Size marker
	1	2	3	Alex	Lud	Ver	Nepalese landraces (56 samples)	

Gel-B (62 samples)

Size marker	Internal std.							Size marker
	1	2	3	Alex	Lud	Ver	Nepalese (51) + Canadian (5) (56 samples)	

Figure 5 Sample plan for two gels (A and B) used to analyze 115 barley samples and internal standards. The first three lanes (1, 2 & 3) were designated for internal standards. The German cultivars, Alexis (Alex), Ludmilla (Lud) and Verena (Ver) were used as reference genotypes to control the allele size variation between the two gels

2.2.3 Statistical analysis

The SSR alleles were scored into binary codes (0 or 1) and similarity coefficients (DICE) between the genotypes were computed as described in the section 2.1.3.1. The UPGMA-clustering was performed and goodness-of-fit of the clustering was

determined by correlating the original similarity matrix with cophenetic values expressed on each node of the dendrogram according to the Mantel test procedure (Mantel 1967) with the software NTSYS-pc version 2.1 (Rohlf 2000) (Exeter Software, Setanket, N.Y.). The gene diversity estimated at each SSR locus and the mean diversity across the loci (diversity Index, DI) followed the equation described by Nei (1978) (section 2.1.3.1).

2.3 Disease reaction of Nepalese hulless barley landraces

The barley landraces used for the molecular analysis (Table 3) were evaluated for the reaction to barley mild mosaic virus, leaf rust and powdery mildew.

2.3.1 Test for Barley mild mosaic virus (BaMMV) resistance

Ten plants were evaluated for each accession in two separate tests comprising 5 plants each following the mechanical infection procedure described by Friedt (1983). Plants were infected with BaMMV in the greenhouse at the 4 to 5 leaf stage using the BaMMV-inoculums extracted with a Pollähne sap-press (http://www.meku-pollaehne.de/) from the leaf material of BaMMV-infected barley *cv* Gerbel. The sap was diluted (1:10) in K_2HPO_4 buffer (0.1 M; pH 9.1), and carborundum powder (mesh 300) was added (0.5 g/25 ml). The mechanical inoculation was carried out by a spray gun (Sata Dekor/ Z−Universal) using 8−bar pressure (air compressor) and a nozzle set with a 0.5 mm diameter. The youngest and the second youngest leaf of each plant were sprayed from both sides with an average of 2.5 ml of diluted sap. The inoculums were kept cool (+4ºC) during the preparation and mechanical infection. After inoculation, plants were briefly rinsed with tap water and kept inside the shade for 24 h at +18ºC; subsequently plants were grown in the greenhouse under natural light conditions at approximately +16ºC. The infected plants were examined serologically after 4 weeks using DAS-ELISA according to Clark and Adams (1977). The optical density was estimated photometrically at a measurement wavelength of 405 nm and a reference wavelength of 620 nm (Easy Reader 400 ATX, SLT Lab instruments, Crailsheim).

2.3.2 Test for powdery mildew (*Blumeria graminis* f. sp. *hordei*) reaction

Twenty seeds of each accession were sown in a plastic cone and grown at 18-22 °C in a greenhouse. Plants were inoculated with the isolates 217 (D12/12) and 178 (D40/4), when the first leaf was fully expanded (10 to 14 days after the sowing).

Table 9 German and exotic barley cultivars used in the study

1. German/exotic collection (Stoll et al. 2002)	Cultivars	Origin\Breeder
German cultivars (1984-1999)	Tessy	Streng
	Ludmilla[a]	Firlbeck
	Alexis[a]	Breun
	Verena[a]	Hadmersleben
	Carola	Nordsaat
	Opal	Nickerson
	Tokyo	Nickerson
	Theresa	Secobra
	Cita	Carsten/Eger
	Hanna	Bauer
	Jana	Dippe/Momont
	Angora	Breun
	Marinka	Cebeco
	Danilo	Lochow
	Andrea	Eckendorf
German cultivars (1953-1980)	Tapir	Semundo/HAEG
	Franka	Streng
	Gerbel	Lochow
	Igri	Ackermann
	Birgit	Borries-Eckendorf
	Sonja	Engelen
	Malta (1970)	Ackermann
	Malta (1968)	Ackermann
	Vogelsanger Gold	Hauptsaaten
	Dura	Streng
	Mädru	Borries-Eckendorf
	Hauters Wintergerste	Schmidt
German cultivars (1891-1932)	Mahndorfer	Wulffen
	Vogel Agaer	Vogel
	Peragis Mittlefrüh	Peragis
	Mausberg	Dr. Mausberg
	Tschermarks	Ackermann
	Mammuuth	Borries-Eckendorf
	Friederichswerther Berg	Meyer
	Derenburger	Derenburger
Exotic collection	Mokusekko 3	China
	Taihoku A	Taiwan
	Muju covered 2	Korea
	Chikurin Ibaraki 1	Japan
	Ea 52	Japan
	Misato Golden	Japan
	Resistant Ym No. 1	Japan
	Bulgarian 347	Bulgaria
	Russia 32	Russia
	Russia 57	Russia
	Krasnodar 1920	Russia
	Anson Barley	USA
H. spontaneum accessions	09-01	Israel
	09-09	Israel
2. Naked barley cultivars	Alamo	Canada
	Candle	Canada
	Freedom	Canada
	McGwire	Canada
	Silky	Canada

a, Not included in the set of German/exotic collection (Stoll et al. 2002)

The infection was carried out by gently shaking the pots with powdery mildew infected plants. This facilitates the thorough distribution of conidia on the test plants. To avoid mixing of individual strains, inoculation was carried out separately. The inoculated plants were placed in a climatic chamber (18°C, night 15°C). The plants were assessed 12−14 days after the inoculation using a 0−4 scale (Jahoor and Fischbeck 1987). The plants with 0, 1 and 2 are scored resistant, whereas 3 and 4 are susceptible. Tests were carried out at the Institute of Epidemiology and Resistance resources, Aschersleben.

2.3.3 Test for leaf rust (*Puccinia hordei*) reaction

Five seeds of each accession were sown in a plastic cone and grown at 19−25 °C in a greenhouse. Plants were inoculated with the isolate J 80, when the first leaf was fully expanded, i.e., about 10 to 14 days after sowing. Prior to inoculation, plants were sprayed with water plus Tween 20 solution. For the infection, pots with infected plants were shaken gently over the test plants in order to evenly distribute the spores. The inoculated plants were placed in a growth chamber (20−22°C) under a cover to ensure maximum atmospheric humidity. After 24 hours of mist period, plants were grown under a normal greenhouse condition. The test plants were scored 12−14 days after the inoculation using a 0−4 scale (Levine and Cherewick 1952). The scores 0, 1 and 2 indicate resistance (low infection type), whereas 2-3, 3 and 4 indicate compatible reactions (high infection type). Tests were carried out at the Institute of Epidemiology and Resistance resources, Aschersleben.

2.4 Genetic studies on BaMMV resistance in Nepalese hulless barley landraces

The study was carried out to obtain preliminary information on genetics of BaMMV resistance in Nepalese hulless barley germplasm.

2.4.1 Allelism test for BaMMV resistance in selected Nepalese landraces

Crosses were made between selected BaMMV resistant landraces during spring 2004 at the Experimental Station of the Institute of Crop Science and Plant Breeding I, Giessen (Table 10). The F_1 seeds were multiplied during the winter 2005 in the greenhouse (IPZ, Giessen) ensuring self-pollination and F_2 seeds were obtained. The F_2 progeny plants were tested for reaction to BaMMV. In total 30 plants of each cross progeny were mechanically infected with BaMMV and assessed for the presence of virus particles using DAS-ELISA as described in the section 2.3.1.

2.4.2 Identification of genes conferring BaMMV resistance in Nepalese hulless barley

This was performed by F_1 progeny analysis of crosses between the resistant Nepalese landraces and German or exotic barley cultivars carrying known genes conferring BaMMV resistance. The hybridization plan is presented in Table 11. The Nepalese landraces were selected from diverse geographic origins.

Table 10 BaMMV−resistant landraces used for the allelism test

1. Annapurna BC-1 x Pisang-8	5. Annapurna BC-1 x Ngyak-4
2. Annapurna BC-1 x Pisang-4	6. Sipche-2 x Tilman Camp-1
3. Thangja-2 x Chame-12	7. Sipche-2 x Sikha-7
4. Pisang-4 x Pisang-8	8. Annapurna BC-2 x Pisang-4

The crosses were made during the winter 2004/05 in the greenhouse, see above IPZ (Giessen). To synchronize flowering, Nepalese landraces were sown in two different dates in a week interval, whereas German or exotic cultivars were sown in four different dates. This followed one week before the first sowing of the female parents and in weekly intervals thereafter. The F_1 seeds were harvested during spring 2005, and tested for BaMMV susceptibility in the greenhouse during winter 2006 following the mechanical infection procedure described in the section 2.3.1. In total 5 to 10 plants for each cross were evaluated depending on the availability of F_1 seeds.

Figure 6 Barley plants grown in the greenhouse (IFZ Giessen); heads bagged after emasculation and pollination

Table 11 BaMMV-resistant Nepalese landraces and German or exotic barley cultivars carrying known genes conferring resistance to BaMMV used in the hybridization. The symbol “x” indicates crosses carried out

Female\Male[a] parents	*rym2*	*rym3*	*rym4*	*rym5*	*rym8*	*rym9*	*rym11*	*rym12*	*rym15*
AnnapurnaBC-1 (1/N615)	x	x	x	x	x	x	x	x	x
TilmanCamp-1 (116/N009)		x	x	x	x	x		x	x
Bimtakothi-10 (9/N019)	x		x	x	x	x	x		x
Thonje-5 (106/N602)		x	x	x	x	x	x	x	x
Pisang-4 (54/N323)	x		x	x	x	x		x	x
Pisang-8 (58/N652)				x	x	x	x		x
Thangja-2 (94/N318)	x	x		x	x	x	x	x	x
Sama-8 (69/N324)	x	x	x	x	x	x	x	x	x
Sipche-2 (81/N377)	x	x	x	x	x	x	x	x	x
Chame-8 (15/N045)			x		x		x	x	
Chame-12 (18/N349)		x	x	x	x	x	x	x	x
Jomson-2 (28/N673)			x	x	x	x	x		x
Ghara-2 (23/N050)				x	x	x	x	x	
Sikha-7 (78/N355)	x	x	x	x	x	x	x		

a, Carrier of different BaMMV resistance genes: *rym2*=MihoriHadaka (Japan); *rym3*=Ishukushirazu (Japan); *rym4*=Express (Germany); *rym5*=W550/412.1/4, W550/412.1/4, W575/333/1, W575/333/2, W548/178/4 (All Germany); *rym8*=10247 (Yugoslavia); *rym9*=Bulgarian347 (Bulgaria), 1132.2/4/2 (Germany); *rym11*=Russia57 (Russia), 1289.1/2/29/2 (Germany); *rym12*=Muju covered 2 (Korea); *rym15*=Chikurin Ibaraki x Igri-4/17 (Germany)

In total 20−25 florets per head were emasculated and covered with a glassine bag and pollinated after 2 to 3 days by inserting matured anthers into the florets. The pollinated heads were tagged and covered with the glassine bag until harvest.

3. Results

3.1 Genetic diversity and population differentiation of Nepalese hulless barley landraces

3.1.1 Allelic diversity and polymorphism information content of the SSR markers

Out of the 44 SSRs analyzed, 41 turned out to be polymorphic and three monomorphic (HvHVA1, HvLOX and HVLEU). Residual heterozygosity was observed for in total 6 genotypes at 5 different marker loci (Bmag0223, Bmac0029, Bmac0399, Bmac0093 and HVM62). The accessions N-6 and Nepal-7 had the highest number of heterozygous loci (4), whereas accessions Pisang-5, Pisang-6, Pisang-7 and Pisang-8 showed double bands only for Bmac0399. The number of alleles and PIC values of SSRs are given in Table 12. The 41 polymorphic SSRs resulted in 227 alleles averaging 5.54 alleles per locus. The highest number of alleles (17) in polymorphic SSRs was scored for Bmac0156 and the lowest (2) for WMC1E8, HvMLO3, EBmac0970, Bmac0040, Bmac0316, Bmac0218 and HVMCA. The highest PIC value was estimated for Bmac0273 (0.88) and the lowest for Bmac0040, Bmac0316 (0.02), with a mean of 0.50 on the 41 polymorphic SSRs. The markers Bmac0154, Bmac0032, Bmac0113, Bmag0223, Bmac0273, Bmag0007 and Bmac0156 demonstrated a high level of allelic diversity and were found to be most informative (PIC >0.80). A strong positive correlation (r=0.76) was estimated between the PIC values and the number of alleles detected at each marker locus.

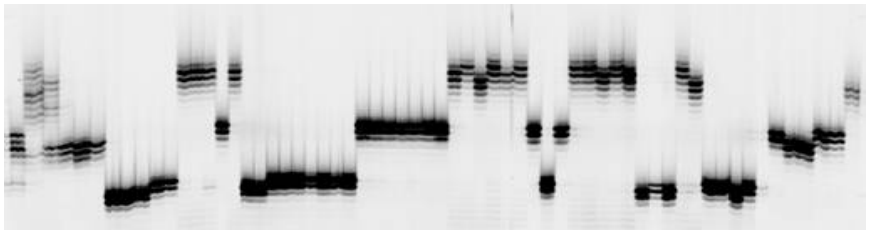

Figure 7 Alleles profile of SSR marker Bmac0113 analyzed on a sample of Nepalese hulless barley landraces

The PIC values of the SSRs averaged on each of the 7 chromosomes, and compared between Nepalese landraces and the work of Macaulay et al. (2001) revealed that these values are highly comparable for the chromosomes 4H (0.477 *vs* 0.567), 5H (0.582 *vs* 0.517) and 7H (0.657 *vs* 0.648), whereas mean PIC values for

the chromosomes 1H (0.596 *vs* 0.684), 2H (0.487 *vs* 0.660) and 3H (0.477 *vs* 0.715) on Nepalese landraces are slightly less than that observed in Macaulay's experiment. A contrasting difference was found for chromosome 6H in this respect with a mean PIC=0.266 on Nepalese landraces and mean PIC=0.686 reported by Macaulay et al. (2001).

3.1.2 Genetic relatedness of Nepalese hulless barley landraces

The 44 SSRs uniquely fingerprinted the landraces except Sipche-4, Sipche-6, Sipche-7 and Sipche-9. The DICE similarity coefficients varied from 0.24 to 1.00 with an overall mean of 0.50 (Appendix-I). The minimum similarity was observed between the genotypes of Sama *vs* Pisang, and Thomje *vs* Pisang. Comparing the mean genetic similarity between landraces within the geographic region, those derived from Sama exhibited the minimum similarity (mean DICE=0.53, range: 0.27-0.93) followed by Ngyak (mean DICE=0.57, range: 0.40-0.89) indicating diverse landraces. In contrast to this, a high genetic similarity was observed between the landraces of Sipche (mean DICE=0.87, range: 0.61-1.00).

The UPGMA cluster analysis revealed two broad groups of landraces (Figure 8): A small cluster consisted of 13 landraces from Philem, Sama, Thomje and Sipche. While all the others (94 landraces) are grouped in a big cluster. However, the landraces can be seen in 10 distinct groups at sub-cluster level. The largest group (I) consisted of 26 landraces that are from Annapurna BC, Bimtakothi, Chame, Pisang, Thangja, Thonje and Tilman camp. In this group, all the genotypes from Bimtakothi except Bimtakothi-13 are clustered. However, landraces of other origins did not show consistency in clustering. The group (II) consisted of five genotypes, three from Sama, and landraces Ngyak-11 and Lih-Dharna-Gal.

Similarly, group (III) consisted of all the landraces of Pisang origin, except Pisang-4. In contrast to this, group (IV) represents landraces of diverse origin (Bimtakothi, Sipche, Nepal and Chame); however, within this group, landraces are well separated according to their origin. Likewise, group (V) also comprised landraces of different origins (Gho, Ngyak, Tsumje and Pork) but unlike in group (IV) the sub-clusters are formed irrespective of the origin.

Table 12 Chromosomal locations, number of alleles detected and polymorphism information content (PIC) of 44 SSRs analyzed on 107 hulless barley landraces

SSRs	Chromosome	Nos. of alleles	PIC	
			Nepalese landraces	Macaulay et al. (2001)
Bmac0399	1H	7	0.67	0.85
Bmac0154[a]	1H	10	0.80	
Bmac0032	1H	15	0.82	0.75
Bmag0211	1H	8	0.62	0.75
HvHVA1	1H	1	0.00	0.63
WMC1E8	1H	2	0.07	0.44
Bmac0093	2H	5	0.60	0.71
Bmac0134	2H	3	0.44	0.79
Bmag0378	2H	3	0.34	0.50
EBmac0415	2H	3	0.60	0.66
HVM36	2H	4	0.41	0.54
HVM54	2H	4	0.53	0.76
Bmac0029[a]	3H	7	0.71	
Bmac0067	3H	7	0.75	0.78
Bmac0209	3H	4	0.38	0.84
Bmag0013	3H	8	0.26	0.81
Bmag0136	3H	3	0.04	0.40
Bmag0225	3H	4	0.65	0.9
HVM62	3H	4	0.55	0.56
Bmag0353	4H	6	0.66	0.55
Bmag0384	4H	4	0.70	0.67
EBmac0701	4H	4	0.47	0.74
HVM40	4H	4	0.40	0.59
HVM67	4H	5	0.30	0.50
HvMLO3	4H	2	0.33	0.35
Bmac0113	5H	9	0.83	0.79
Bmag0222	5H	3	0.60	0.62
Bmag0223	5H	10	0.81	0.90
EBmac0684	5H	6	0.60	0.67
EBmac0970	5H	2	0.07	0.08
HvLOX	5H	1	0.00	0.08
HVLEU	5H	1	0.00	0.48
Bmac0018	6H	3	0.44	0.59
Bmac0040	6H	2	0.02	0.94
Bmac0316	6H	2	0.02	0.70
Bmag0009	6H	5	0.51	0.47
Bmag0218	6H	2	0.22	0.65
EBmac0806	6H	4	0.39	0.77
Bmac0273	7H	11	0.88	0.59
Bmag0385[a]	7H	5	0.54	
Bmag0007[a]	7H	14	0.83	
Bmag0120	7H	4	0.53	0.75
Bmac0156	7H	17	0.87	0.84
HVCMA	7H	2	0.29	0.41
Mean		5.54[b]	0.50[b]	0.64

a, Not included in the set of SSRs proposed by Macaulay et al. (2001)
b, Calculated on 41 polymorphic loci

All the landraces of the KaliGandaki (KG) region can be found in groups (VI) and (VII). Within the group (VI), genotypes from the upper basin of KG (Jomson, Kagbeni and Tukucha) formed a distinct sub group, whereas genotypes of different origin, e.g. that of Chame and Thonje did not converge in clustering. Similarly, group (VII) comprised all the landraces of the lower basin of KG (Ghara, Sikha and Ulleri) except the landrace Phalatey which is placed in group (VI), and three other accessions, Nepal-1, Nepal-2, and Solu Uwa. The group (VIII) consisted of two genotypes, N-6 and N-12 of unknown origin, and group (IX) consisted of three genotypes of Philem origin. Moreover, group (X) comprised of landraces of diverse origin (Sama, Thomje and Sipche) and none of the sub-clusters in this group represents a specific origin. In general, landraces of common origin are clustered in smaller groups and some of the clusters represent a broad geographic region (Figure 8).

3.1.3 Model based groups

The 107 landraces were assigned into nine genetically distinct groups (Figure 9). Due to large test population and many SSR loci used, the STRUCTURE software required fairly long runs (50,000 burn-in and 100,000 iterations after burn-in) to achieve consistent results. In a range of simulated runs for presumed number of populations (k) =1 to 12, the most appropriate number of populations (k) was identified at (k) =9, where the natural log probability of the data which is proportional to the posterior probability was maximized (-3516.6). Each of the 9 groups identified with the structure analysis was given a name either after the most frequent origin found in the group or the geographic region representative of the landraces in the group.

The mean inferred ancestry (%) of 9 groups and corresponding geographic origins are given in Table 13 (inferred ancestry of individual genotype is given in Appendix-III). The groups Sikha (1), Nepal (2), Pisang (6), Thonje (7) and Sipche (9) conferred a high mean inferred ancestry (>90 %) and distinct populations were identified from each of these groups. Contrarily, groups Ngyak (3), Sama (4) and Jomson (5) had <90 % mean inferred ancestry, however possessed a number of landraces with ≥90 % common inferred ancestry and therefore comprised distinct populations.

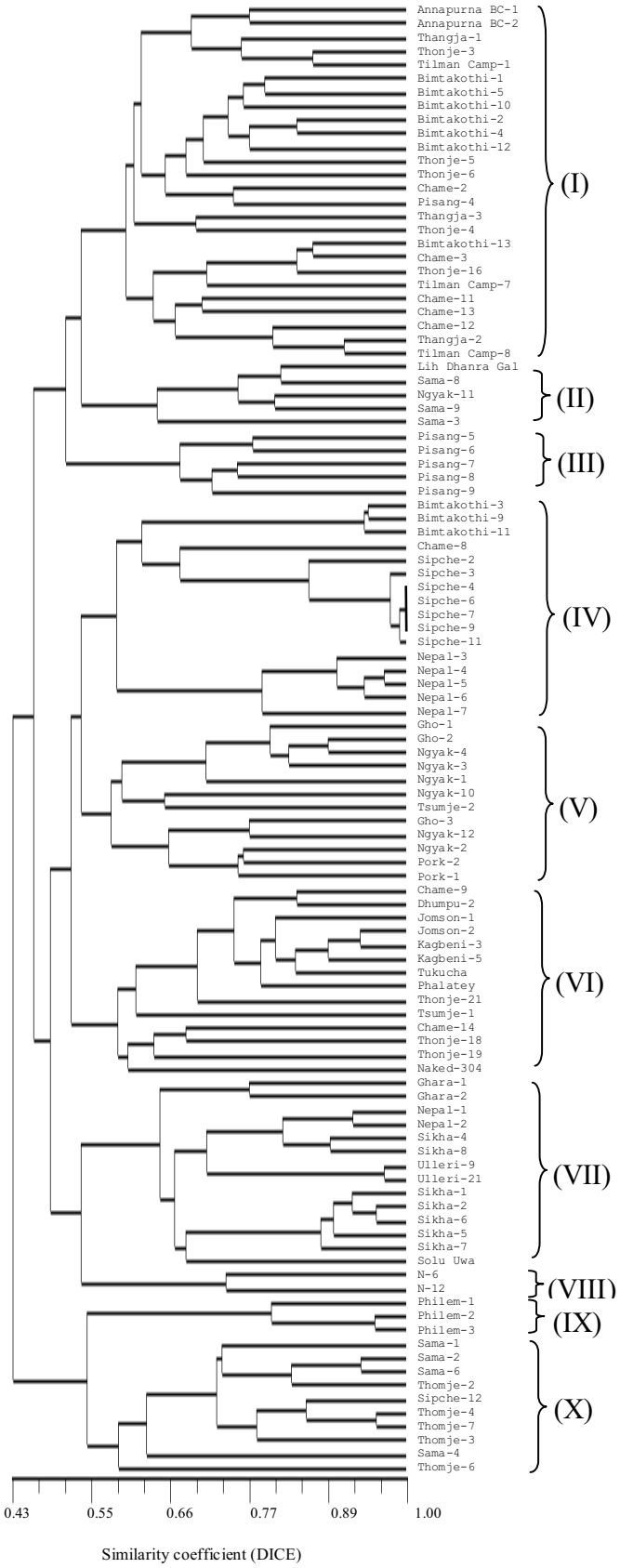

Figure 8 UPGMA clustering dendrogram of 107 Nepalese hulless barley landraces based on the genetic similarity coefficients. Sub-clusters are marked with Roman numerals (I-X) (Appendix-II)

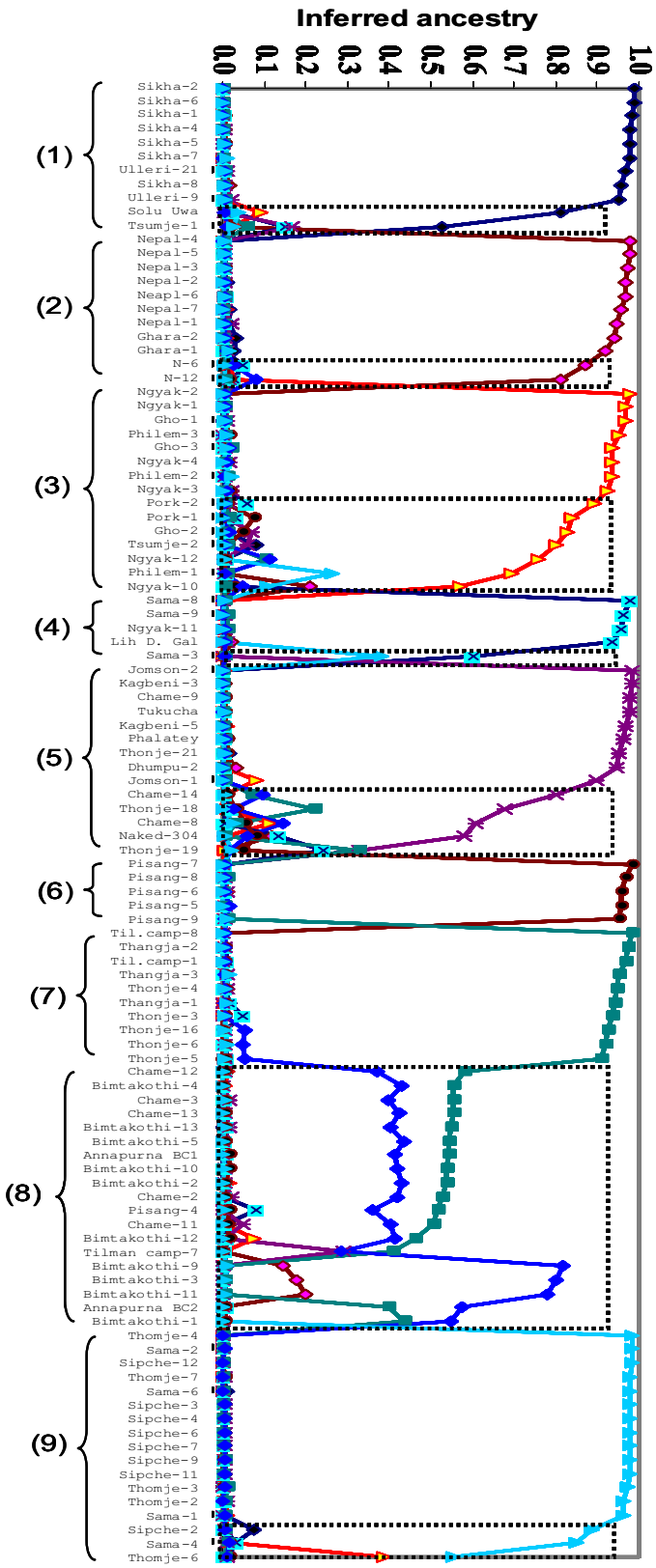

Figure 9 Inferred ancestry of 107 landraces and the 9 model based groups (1-9) identified with the STRUCTURE software. The light areas connected by the graphic lines indicate distinct populations and rectangles denote admixtures (Appendix-IV)

This way, eight distinct populations specific to geographic region were identified. These populations consisted of landraces from the respective geographic regions with some exceptions. For instance, Sama-1, Sama-2 and Sama-6 belong to the Sipche population (9) instead of the Sama population (4). Similarly, Chame-9, Thonje-21 and Phalatey are found in the Jomson group (5), and Ngyak-11 is found in the Sama population (4). However, although the geographic locations of Thomje, Thangja, and Tilman camp are not indicated in the map (Figure 4) because of some doubts, the landraces of Thomje that are found in the Sipche group (9), and landraces originated from Thangja and Tilman camp found in the Thonje group (7) are considered to be the populations of the Sipche and Thonje region, respectively.

In total 36.5 % of the landraces were found with a mixed ancestry, and all the landraces in group (8) turned out to belong to this category. In this group, the mean inferred ancestry corresponding to the origin (Bimtakothi) is only 48.08 % (Table 13) and a significant proportion is shared with Thonje origin (43.25 %) which is close to Bimtakothi (Figure 4). Likewise, groups Sikha, Ngyak, Thonje and Sipche shared the largest proportion of mixed ancestry with Jomson (2.00 %), Sipche (2.93 %), Bimtakothi (1.92 %) and Ngyak (2.67 %), respectively.

This shows an affinity in shared ancestry between the geographically closest populations. However, exception existed for Jomson that shared 4.81 % mixed ancestry with Thonje and 3.14 % with Sama. Similarly, Sama had the largest mixed ancestry (7.78 %) with Sipche surpassing the nearest group Ngyak (0.30 %). The landraces of Pisang (group 6) shared a minimum mixed ancestry (<1.00 %) to any other origin.

3.1.4 Comparing UPGMA clusters and the model based groups

The genetic similarity (GS) based UPGMA clusters (Figure 8) and the model based (MB) groups (Figure 9) are compared. All the landraces of Bimtakothi are placed in MB group (8) by the structure analysis, whereas three landraces namely, Bimtakothi-3, Bimtakothi-9 and Bimtakothi-11 are separated from the others in the UPGMA clustering (GS groups I and IV). The MB approach better elucidated the GS group (I), out of which landraces from the Thonje region formed a distinct group, while all the others are found to be mixtures. The GS groups (II) and (III) are consistent with MB groups (4) and (6), respectively. Similarly, GS groups (V), (VI) and (VII) can be compared to MB groups (3), (5) and (1), respectively. The accessions found in these

groups are the same with some exception. For example, the MB group (5) consisted of an additional landrace (Chame-8) and lacks (Tsumje-1) compared to the corresponding GS group (VI). The accession Tsumje-1 is placed in the MB group (1). Moreover, landraces Ghara-1, Ghara-2, Nepal-1 and Nepal-2 that are found in the GS group (VII) are not in the respective MB group (1). The MB group (1) lacking these landraces better represents the distinct population of the Sikha region. The GS groups (VIII), (IX) and (X) converged into MB groups (2), (3) and (9), respectively. The MB groups (3) and (9) having the landraces of GS groups (IX) and (X) represent the broader geographic region of Ngyak and Sipche, respectively.

Table 13 Origin and mean inferred ancestry (%) of the nine model based groups identified with the STRUCTURE software (Pritchard et al. 2000)

Groups\ Origins	Mean inferred ancestry (%)								
	Sikha	Nepal	Ngyak	Sama	Jomson	Pisang	Thonje	Bimtakothi	Sipche
(1) Sikha	91.85	0.75	1.21	1.77	2.00	0.42	0.75	0.37	0.86
(2) Nepal	1.15	93.79	0.84	0.95	0.79	0.47	0.33	1.30	0.41
(3) Ngyak	0.96	2.09	86.50	1.08	1.69	1.59	1.41	1.69	2.93
(4) Sama	0.26	0.82	0.30	88.68	0.48	0.44	0.80	0.44	7.78
(5) Jomson	0.39	1.47	1.86	3.14	83.17	1.96	4.81	2.65	0.56
(6) Pisang	0.28	0.36	0.46	0.46	0.56	96.38	0.56	0.66	0.32
(7)Thonje	0.38	0.31	0.34	1.16	0.44	0.38	94.51	1.92	0.60
(8) Bimtakothi	0.31	3.14	1.01	0.94	2.31	0.66	43.25	48.08	0.29
(9) Sipche	0.96	0.43	2.67	0.42	0.42	0.38	0.39	0.45	93.94

3.1.5 Genetic diversity and population differentiation

Out of the eight populations determined by structure analysis, 7 populations of sample size ≥ 5 were selected for genetic diversity and differentiation study (Table 14). The Sama population that comprised only four landraces was not included in the analysis, therefore. The 64 landraces in the 7 populations, representing approx. 60 % of the whole set of 107 landraces studied, exhibited 40 polymorphic SSRs (97.56 %). Likewise, the overall DI of the 7 populations was estimated at DI=0.539 in comparison to DI=0.536 for the whole sample (107 landraces). The SSR marker Bmac40 turned out to be monomorphic on the set of 64 landraces.

The populations varied for polymorphic loci, mean number of alleles per locus, number of unique alleles and for genetic diversity (Table 14). The highest variation in terms of polymorphic loci and mean number of alleles per locus was observed for Ngyak (75.0 %, 2.5) and Thonje (72.5 %, 2.6), whereas Pisang (52.5 %, 2.43), Sikha (60.0 %, 2.25) and Jomson (55.0 %, 2.46) were less variable in this respect. The populations Thonje and Pisang demonstrated the highest numbers of unique alleles (17 and 14, respectively) whereas populations Nepal and Ngyak had the lowest numbers of unique alleles, i.e., 3 and 5, respectively. The numbers of unique alleles detected for Sikha, Jomson and Sipche were comparable (Table 14). The highest genetic diversity was estimated for Pisang (DI=0.559). The DI estimated for Pisang is higher than that of the sub set of 64 landraces or for the whole sample. A comparable level of genetic diversity was observed between populations Nepal (DI=0.494) and Ngyak (DI=0.498), and between Thonje (DI=0.489) and Sipche (DI=0.480). The populations Sikha and Jomson revealed minimum genetic diversity compared to all the 7 populations, i.e., DI=0.435 and DI=0.430, respectively.

Table 14 Sample size, mean inferred ancestry (%), polymorphic loci (%), mean number of alleles per locus, number of private alleles and genetic diversity index (DI) of 7 populations studied for diversity and differentiation

Populations	Sample size	Mean inferred ancestry (%)	Polymorphic loci[a] (%)	Mean nos. alleles per locus	Nos. private alleles	DI
Sikha	9	97.56	60.0	2.25	10	0.435
Nepal	9	96.11	65.0	2.50	3	0.494
Ngyak	8	95.13	75.0	2.50	5	0.498
Jomson	9	96.11	55.0	2.46	8	0.430
Pisang	5	96.40	52.5	2.43	14	0.559
Thonje	10	94.50	72.5	2.60	17	0.489
Sipche	14	97.71	72.5	2.50	10	0.480
Overall	64			4.46		0.539

a, With respect to 40 polymorphic loci found in the set of 64 genotypes

The genetic relationship among the 7 populations is displayed in an unrooted tree diagram with two broad groups (Figure 10). The populations Pisang, Ngyak, Thonje and Jomson are in one group while Sikha, Nepal and Sipche formed a separate group. The population Jomson in the first and Sipche in the latter group are,

however, more distinct from the others. In a more specific interpretation, landraces from the upper basin of rivers Marshyangdi and BudhiGandaki corresponding to the populations Pisang, Thonje and Ngyak are genetically closer. Similarly, landraces from the lower basin of KaliGandaki (population Sikha) and that of population Nepal (location not known) are also closely related, whereas the landraces from upper basin of the river KaliGandaki, i.e., population Jomson, and population Sipche derived from the East of BudhiGandaki are more independent.

The population differentiation parameters θ and R_{ST} were estimated at 0.433 and 0.445, respectively. The pairwise population differentiation and significance tests are shown in Table 15. Out of 21 pairwise combinations among the 7 populations, 15 conferred a significant differentiation. Thonje differed from all the other populations, whereas Sipche, Jomson and Nepal differed from all the others except population Pisang. Similarly, Sikha was differentiated from Nepal, Jomson, Thonje and Sipche, and Ngyak was differentiated from Nepal, Jomson, Thonje and Sipche. The population Pisang however, differentiated only from the Thonje. The linear relationship test between coefficient of genetic differentiation (pairwise θ) and geographic distance revealed a non-significant correlation coefficient (r=0.224, p >0.05) rejecting the hypothesis of isolation by distance.

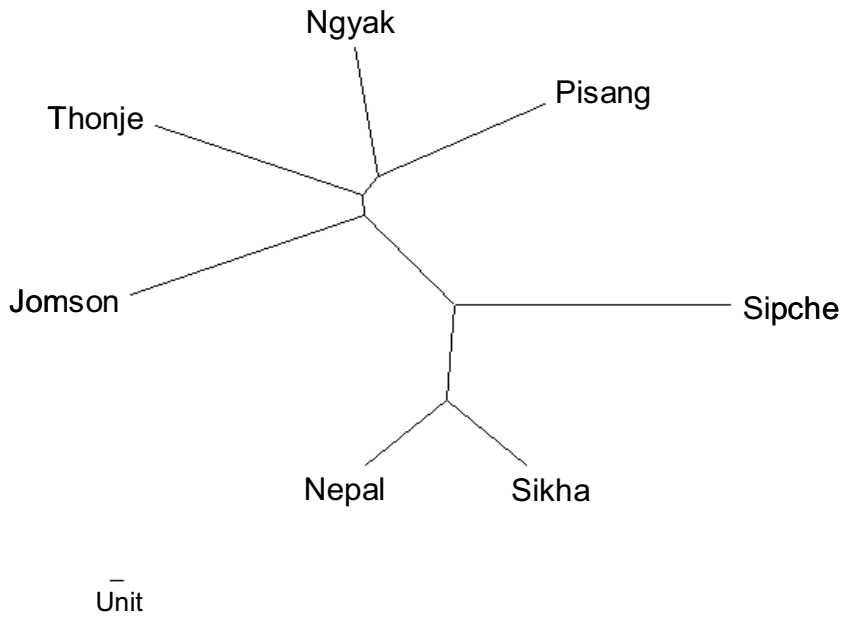

Figure 10 Genetic relationship among the 7 geographic populations revealed with UPGMA clustering on genetic distances (Nei 1978) and displayed as an un-rooted tree diagram

Table 15 Population differentiation measured by pairwise *θ*

Populations	Sikha	Nepal	Ngyak	Jomson	Pisang	Thonje
Nepal	0.310*					
Ngyak	0.473^{ns}	0.335*				
Jomson	0.542*	0.435*	0.387*			
Pisang	0.588^{ns}	0.491^{ns}	0.321^{ns}	0.507^{ns}		
Thonje	0.520*	0.443*	0.335*	0.455*	0.345*	
Sipche	0.462*	0.348*	0.349*	0.476*	0.509^{ns}	0.420*

The *θ* calculated for all the population-pairwise combinations and significance test performed by permuting genotypes among populations (1,000 randomizations). Multiple significance tests were performed after sequential Bonferroni corrections

'*' Significant at $P \leq 0.05$; '*ns*' Non-significant

3.2 Genetic relationship of Nepalese hulless barley landraces with East Asian and Western barley cultivars

3.2.1 Allelic diversity, unique alleles and genetic diversity estimates

All 30 SSRs analyzed on 161 barley genotypes were polymorphic resulting in 237 alleles with a mean of 7.9 alleles per locus. The number of alleles, allele size-range (base pairs) and diversity estimates for the SSRs are given in Table 16. The SSR Bmac0032 was the most polymorphic marker with the highest number of alleles (n=24), whereas HvLOX turned out to be the least polymorphic (n=2) on the set barley genotypes analyzed. The diversity index (DI) was estimated at DI=0.606. Very high level of diversity was observed at three marker loci, i.e., Bmac0032, Bmag0223 and Bmag0007 (> 0.80). In contrast to this, markers HvHVA1 and HvLOX exhibited the lowest diversity (< 0.10). The diversity estimated at SSR loci were strongly and positively correlated with the number of alleles detected (r=0.70).

The unique SSR alleles were analyzed within different groups of barley genotypes: (1) Nepalese hulless landraces (n=107), (2) Canadian hulless cultivars (n=5), (3) German hulled cultivars (n=35), (4) East European hulled cultivars (n=4), (5) East Asian hulled cultivars (n=7), and (6) *H. spontaneum* accessions (n=2). Over 40 % of the alleles correspond to a unique origin or set of barley cultivars/accessions (Table 17). The Nepalese landraces possessed the maximum number of unique alleles (n=36) followed by German cultivars (n=21). In total 12 alleles were specific to two *H. spontaneum* accessions. Similarly, eight alleles were found unique to East European

and East Asian barley cultivars, respectively. The Canadian naked cultivars had 7 alleles that are not shared by any other groups.

Table 16 Thirty SSRs analyzed on the set of 161 barley genotypes, and separately on Nepalese landraces and German cultivars. The genomic location, allele size-range, number of alleles and diversity estimates for the SSRs are presented

SSRs[a]	Genomic location	Allele size range (bp)	Nos. of alleles detected			Diversity estimate (Nei 1978)		
			Nepalese landraces[b]	German cultivars[c]	Whole sample	Nepalese landraces	German cultivars	Whole sample
Bmac0399	1H	118-149	6	8	12	0.707	0.576	0.793
Bmac0032	1H	202-263	15	6	24	0.843	0.777	0.907
HvHVA1	1H	115-138	1	2	3	0.000	0.205	0.085
WMC1E8	1H	189-223	2	2	3	0.072	0.504	0.270
Bmac0093	2H	145-161	5	5	6	0.664	0.393	0.699
HVM54	2H	111-162	4	3	6	0.605	0.210	0.746
EBmac0415	2H	227-248	3	3	5	0.641	0.111	0.651
Bmag0136	3H	200-204	3	2	3	0.037	0.109	0.413
Bmac0067	3H	151-179	7	4	10	0.763	0.605	0.768
Bmac0209	3H	174-193	4	4	9	0.430	0.749	0.711
HVM62	3H	241-275	4	5	8	0.631	0.472	0.781
HVM40	4H	144-162	3	3	9	0.490	0.510	0.724
Bmag0353	4H	88-126	6	7	10	0.662	0.775	0.721
EBmac0701	4H	119-179	4	5	13	0.496	0.568	0.748
HvMLO3	4H	231-247	2	2	3	0.421	0.358	0.498
HVM67	4H	103-120	4	5	8	0.347	0.388	0.660
EBmac0970	5H	186-190	2	2	3	0.072	0.345	0.466
EBmac0684	5H	156-187	6	3	8	0.601	0.490	0.735
Bmag0223	5H	132-183	9	9	14	0.828	0.823	0.892
HVLEU	5H	162-168	1	3	3	0.000	0.429	0.201
HvLOX	5H	150-152	1	2	2	0.000	0.109	0.025
Bmac0316	6H	129-169	2	5	6	0.019	0.507	0.461
Bmag0218	6H	188-194	2	1	3	0.256	0.000	0.532
Bmac0018	6H	133-141	3	3	5	0.513	0.623	0.710
Bmag0009	6H	169-180	5	6	8	0.557	0.735	0.722
EBmac0806	6H	156-167	4	5	7	0.407	0.767	0.656
Bmac0040	6H	177-239	2	10	12	0.019	0.802	0.540
Bmag0007	7H	183-225	15	6	21	0.842	0.628	0.873
HVCMA	7H	131-139	2	2	3	0.350	0.474	0.568
Bmag0120	7H	228-266	4	5	10	0.534	0.623	0.623
Over all			4.37	4.27	7.90	0.474	0.506	0.606

a, Details are given in Table 5
b, n=107
c, n=35

To get an in-depth view on the genetic differentiation between Nepalese hulless barley and Western hulled cultivars, SSRs data on 107 Nepalese landraces (NL) and 35 German cultivars (GC) were analyzed in detail (Table 16). Considering both sets of barleys as a whole, all 30 SSRs analyzed were found to be polymorphic with in total 197 alleles. The total number of alleles scored on NL and GC differed slightly (131 *vs* 129, respectively). Between NL and GC, 63 alleles were common which accounts for about 32 % of the alleles of the whole sample. When analyzed separately on NL and GC, it turned out that three SSRs (HvHVA1, HVLEU and HvLOX) on Nepalese landraces and Bmag0218 on the set of German cultivars are monomorphic. Two SSRs namely, Bmac0032 and Bmag0007 scored the maximum (n=15) and six SSRs (WMC1E8, HvMLO3, EBmac0970, Bmac0316, Bmag0218, Bmac0040 and HVCMA) scored the minimum number of alleles (n=2) on NL, whereas Bmac0040 that showed minimum allelic diversity on Nepalese landraces had the highest number of alleles (n=10) on GC. The SSRs HvHVA1, WMC1E8, Bmag0136, HvMLO3, EBmac0970, HvLOX and HVCMA had the lowest number of alleles (n=2) on the set of German cultivars. The DI for Nepalese landraces and German cultivars was estimated at 0.474 and 0.506, respectively.

Moreover, the diversity estimates presented in Table 16 are summarized for each of the 7 barley chromosomes and compared among NL, GC and the whole sample (n=161) (Table 18). Out of the 30 SSRs listed in Table 16, 26 SSRs that are polymorphic on Nepalese and German sets of barleys were used to calculate the mean gene diversity (GD). On the whole sample, the mean GD estimated for all the 7 chromosomes are on the same level (mean GD range, 0.618 to 0.698). Similarly, the mean GD estimated on Nepalese landraces and German cultivars for chromosomes 1H, 3H, 4H, 5H and 7H are also comparable having a difference range of 0.000 to 0.078. However, the mean GD between Nepalese landraces and German cultivars differed very much for chromosomes 2H (0.637 *vs* 0.238) and 6H (0.303 *vs* 0.687).

The proportion of rare alleles (freq. <0.05) on the sample (NL+GC) was high (44.2 %) and almost 90.0 % of the rare alleles correspond to specific group of barleys, i.e., NL or GC (original allele freq. data are presented in Appendix-V). Likewise, over 50 % of the common alleles (freq. >0.05) on the whole sample were found to be group specific.

The highly differentiated SSR alleles between the Nepalese hulless barley and German hulled cultivars are summarized in Table 19. Almost 20 % alleles are highly differentiated. For instance, Bmag0218 possessed two unique alleles, 192 (bp) is dominant in Nepalese landraces (freq.=0.85) but absent in German cultivars, whereas 188 (bp) is highly dominant in German cultivars (freq.=1.00) but does not exist in Nepalese landraces. Similarly, markers HVM40 and Bmac0209 demonstrated 4 alleles on each locus that are highly dominant but group specific (NL or GC) (Table 19).

Table 17 Number of unique alleles detected on different groups of barley

SSRs	Groups of barley cultivars /accessions					
	Nepalese landraces (n=107)	East Asian *cvs* (n=7)	German *cvs* (n=35)	East Euro-pean *cvs* (n=4)	Canadian naked *cvs* (n=5)	*Hordeum spontaneum* (n=2)
WMC1E8	1	-	-	-	-	-
Bmac0399	1	-	3	1	-	1
HvHVA1	-	-	1	-	-	-
Bmac0032	9	2	-	1	-	2
Bmac0093	1	-	-	-	-	-
EBmac0415	1	-	-	1	-	-
HVM54	-	-	1	-	-	-
Bmag0136	1	-	-	-	-	-
Bmac0209	1	-	1	-	1	-
Bmac0067	2	-	-	-	1	-
HVM62	-	-	1	-	-	-
HVM40	1	1	-	-	2	-
Bmag0353	-	1	1	-	-	1
HvMLO3	-	-	-	-	-	1
HVM67	2	-	1	-	-	1
EBmac0701	4	1	1	1	-	2
EBmac0684	2	1	-	-	-	1
HVLEU	-	-	1	-	-	-
EBmac0970	-	-	-	-	-	-
HvLOX	-	-	1	-	-	-
Bmag0223	2	-	1	-	-	1
Bmac0018	-	-	-	-	-	-
Bmag0218	-	-	-	-	-	-
Bmac0316	-	1	1	-	-	-
Bmag0009	-	-	1	-	-	-
EBmac0806	-	-	1	-	1	-
Bmac0040	-	-	2	2	-	-
Bmag0120	1	-	1	1	1	1
HVCMA	-	-	-	-	-	-
Bmag0007	7	1	2	1	1	1
Total	36	8	21	8	7	12

Table 18 Mean genetic diversity detected at 7 barley chromosomes on the whole sample, and separately for Nepalese landraces and German cultivars, resp.

Genomic region (Chromosomes)	Nos. of SSRs analyzed[a]	Mean genetic diversity (Nei 1978)		
		Nepalese landraces (n=107)	German cultivars (n=35)	Whole sample (n=161)
1H	3	0.541	0.619	0.657
2H	3	0.637	0.238	0.698
3H	4	0.465	0.484	0.668
4H	5	0.483	0.520	0.670
5H	3	0.500	0.553	0.698
6H	5	0.303	0.687	0.618
7H	3	0.575	0.575	0.688

a, SSRs polymorphic on Nepalese landraces and German cultivars

Figures 11 and 12 display the allele profiles of the two highly polymorphic SSRs, Bmac0032 and Bmag0007, respectively. The allele 209 (bp) of Bmac0032 is predominant in NL but it is absent in German cultivars, and alleles 214 (bp) and 216 (bp) are common in GC but absent in Nepalese landraces. Similarly, allele 194 (bp) of Bmag0007 which is predominant in GC is less frequent in Nepalese hulless barley. However, one allele of Bmac0032, i.e., 243 (bp), and two alleles of Bmag0007, i.e., 198 (bp) and 190 (bp) are more or less uniformly distributed between the two sets of barley samples (NL and GC).

3.2.2 Genetic relatedness and UPGMA cluster analysis

Genetic similarity indices (DICE) between the genotypes ranged from 0.032 to 1.00 (original similarity data not shown). Except those genotypes that are not differentiated by the panel of 30 SSRs (Figure 13, genotypes represented by vertical bars indicate DICE=1), the maximum similarity (DICE>0.980) was observed between the Nepalese landraces, Bimtakothi-3 *vs* Bimtakothi-11, Nepal-5 *vs* Nepal-6, and Sipche-11 *vs* Sipche-3, -4, -6, -7, -9, respectively. The minimum genetic similarity was found between the German cultivar 'Franka' and Nepalese landraces Gho-1 and Tsumje-2, respectively (DICE=0.032). The DICE similarity indices when averaged for a group of cultivars or accessions, Nepalese landraces had the highest mean DICE (0.570) followed by German cultivars (mean DICE=0.498). The Canadian naked cultivars demonstrated a comparable mean DICE (0.496) with the German cultivars. However, East Asian cultivars had a lower mean DICE value (0.464) compared to German or Canadian cultivars.

Table 19 Highly differentiated SSR alleles between Nepalese landraces and German cultivars

Locus	Alleles (bp)	Allele frequency	
		Nepalese landraces (n=107)	German cultivars (n=35)
Bmag0120	228	-	0.200
Bmac0018	141	-	0.200
	135	0.635	-
	133	0.280	-
Bmag0218	192	0.850	-
	188	-	1.000
Bmac0316	129	-	0.257
HVM40	162	-	0.228
	160	0.635	-
	154	0.327	-
	146	-	0.657
HVMCA	140	-	0.371
	132	0.775	-
Bmac0209	191	-	0.314
	189	-	0.228
	185	0.728	-
	174	-	0.285
Ebmac0684	174	0.579	-
WMC1E8	188	-	0.457
HVLUE	168	-	0.257
HVM67	117	-	0.771
	115	0.785	-
Bmag0009	171	0.626	-
Bmac0399	125	0.411	-
EBmac0415	237	0.457	-
Ebmac0701	147	-	0.628
	134	0.682	-
EBmac0806	165	-	0.371
Bmag0223	174	-	0.314
	167	0.317	-
HVM62	273	0.485	-
HVM54	154	0.500	-
	151	0.364	-
Bmag0007	196	0.289	-
Bmac0032	216	-	0.314
	214	-	0.285
	209	0.336	-
Bmac0040	230	-	0.342

-, Alleles not detected

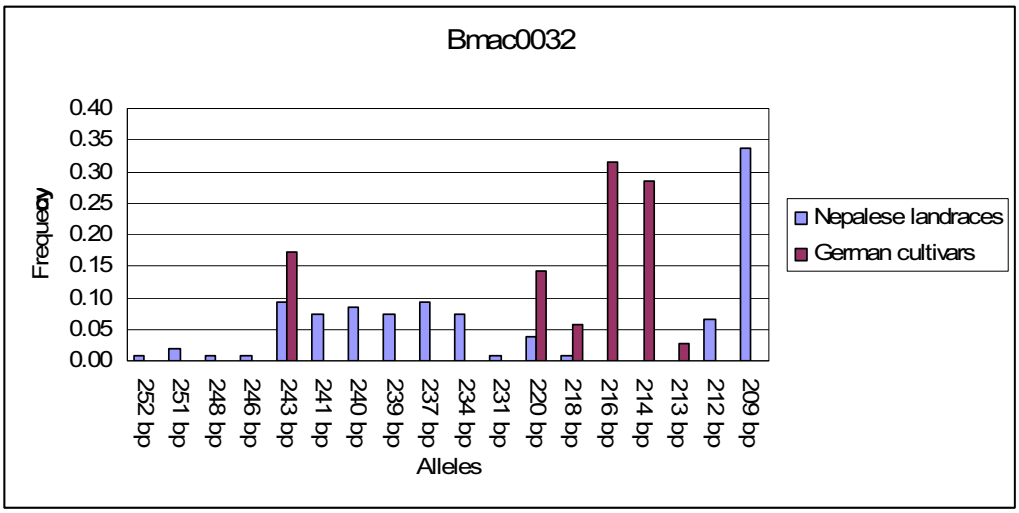

Figure 11 Allele profile of Bmac0032 detected on Nepalese hulless barley and German hulled cultivars

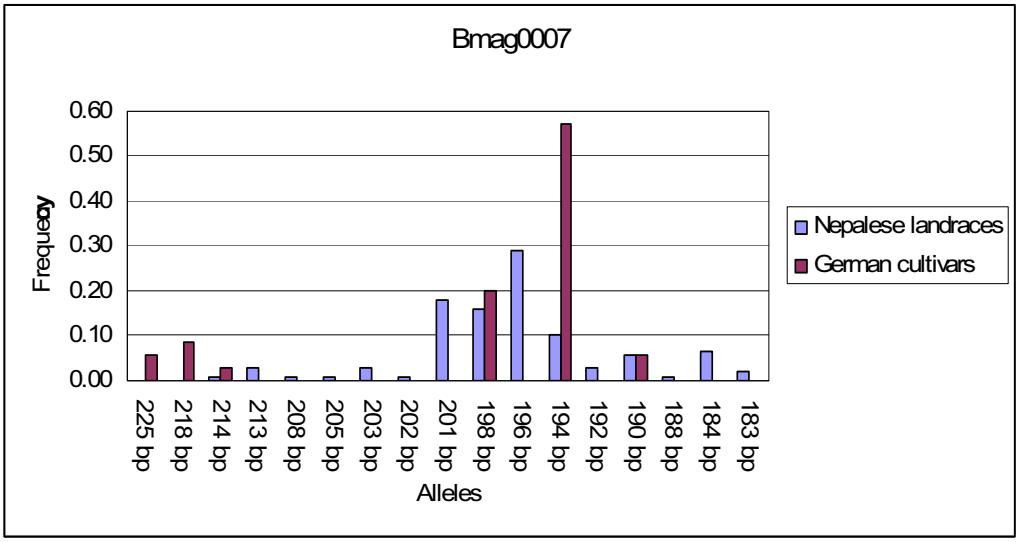

Figure 12 Allele profile of Bmag0007 detected on Nepalese hulless barley and German hulled cultivars

The UPGMA clustering resulted in to two broad clusters and several sub clusters of 161 barley genotypes (Figure 13). The Mantel test (Mantel 1967) revealed that these clusters are in very good fit with the original similarity matrix (r=0.945). The two broad clusters clearly differentiated the barleys from the East and the West. All the Western cultivars along with two *H. vulgare* subsp. *spontaneum* accessions clustered together (Group I). Similarly, Nepalese hulless barley landraces and East Asian cultivars are found to be genetically closer and shared a common broad group (Group II). In this respect, two Japanese cultivars, Misato Golden and Resistant Ym No. 1

exceptionally grouped with the European cultivars. Out of the three accessions derived from Russia, Russia 32 and Krasnodar 1920 grouped with European cultivars, and Russia 57 with the East Asian cultivars.

Besides the two highest order clusters that are in accordance with the broader geographic grouping of the barley genotypes, sub groups within these clusters are also consistent with the origin of barley cultivars/accessions (Figure 13). For example, the Western cultivars/accessions (Group I) are further divided in to three distinct groups that represent Canadian naked cultivars (Ia), European hulled cultivars (Ib) and *H. vulgare* subsp. *spontaneum* accessions (Ic). The group (Ia) comprised all five Canadian naked cultivars and the German cultivar Alexis. In group (Ib) all the European cultivars are clustered, except the German cultivar Alexis (the only spring type in the group) that grouped together with the Canadian cultivars, and Russia 57 (Russia). Additionally, two East Asian cultivars (Resistant Ym No. 1 and Misato Golden) and 'Anson barley' origin to USA are placed within this group. The German cultivars can be seen distinct from the others.

In group (II) Nepalese hulless barley landraces and East Asian hulled cultivars are well differentiated and form two separate sub-groups, (IIa) and (IIb), respectively. Among the East Asian cultivars (IIb), Chinese *cv.* Mokusekko 3 can be seen distinct from the cultivars that are from Japan, Korea or Taiwan. Similarly, Russia 57 (Russia) is distinct from the East Asian barleys in this group (IIb).

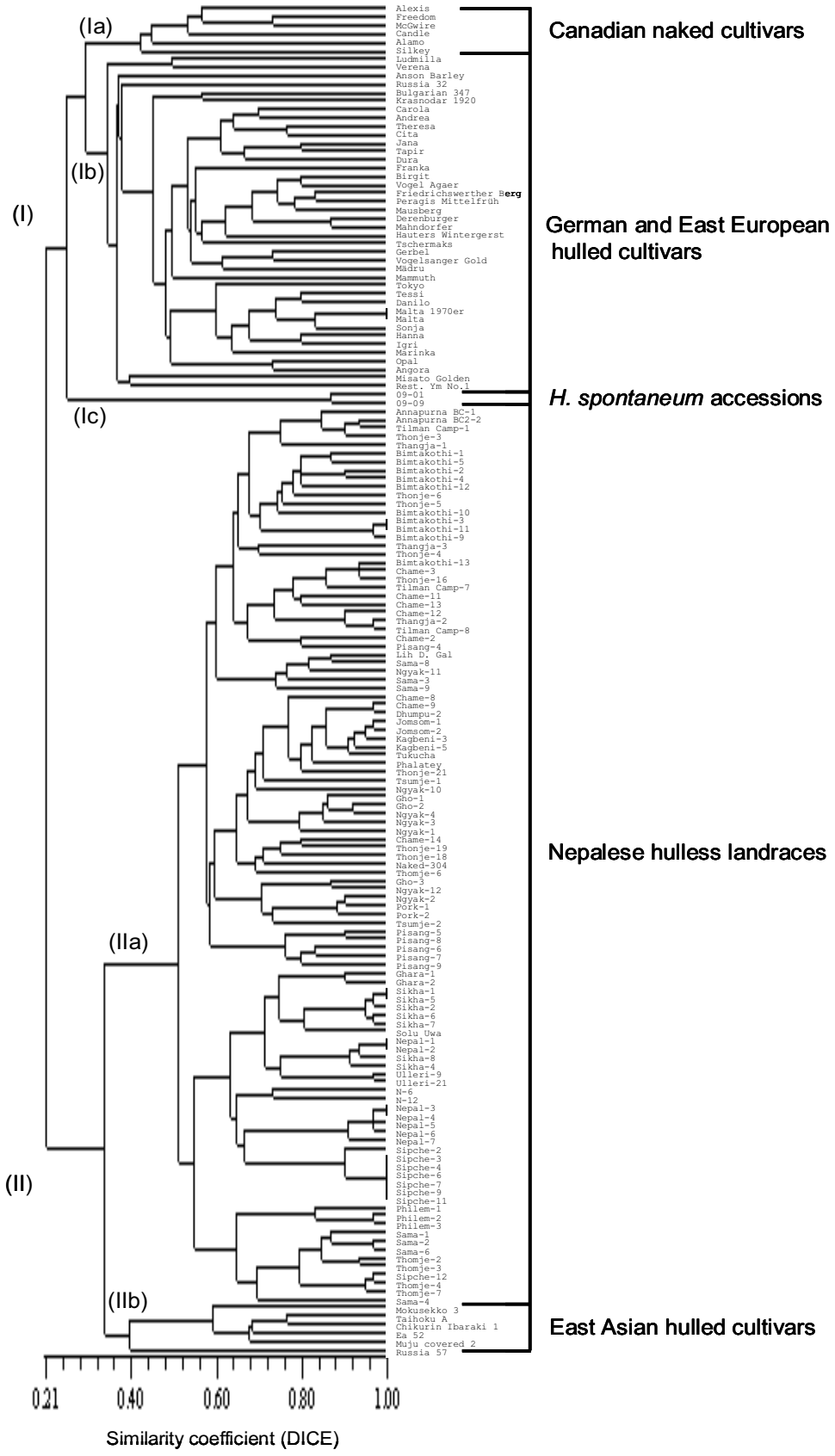

Figure 13 UPGMA dendrogram of 161 barley genotypes analyzed with 30 SSRs (Appendix-VI)

3.3 Disease reaction of Nepalese hulless barley landraces

The results of Barley mild mosaic virus (BaMMV), powdery mildew (*B. graminis* f. sp. *hordei*) and leaf rust (*P. hordei*) tests are presented in Table 20. A high frequency of landraces derived from Sikha, Sipche, Thonje, Chame and Bimtakothi are resistant to BaMMV. Regarding powdery mildew, very few landraces showed a resistance reaction to Isolate 217 (D12/12). However, a number of landraces conferred resistance reaction to Isolate 178 (D40/4). The isolate specific resistance pattern of the landraces compared with that of the standard set of genotypes used for the virulence test (Institute of Epidemiology and Resistance resources, Aschersleben) indicated that Nepalese hulless barley germplasm harbor mainly, We, U2, / St, U2 / Ly, We, Kw, La / Ha / Mlp / Mla22 / Mla27 types of resistance. None of the landraces were found to be resistant to leaf rust Race J 80.

Table 20 Reaction of Nepalese hulless barley landraces to barley mild mosaic inducing BaMMV, powdery mildew (*B. graminis* f. sp. *hordei*) and leaf rust (*P. hordei*)

OU No.	Landraces	Reaction to			
		BaMMV	Powdery mildew		Leaf rust
			Isolate 217	Isolate 178	Race J 80
N615	Annapurna BC1	R	3	0	3
N015	Annapurna BC2	R	3	2	3
N303	Bimtakothi-1	S	4	1	3
N603	Bimtakothi-2	S	-	-	-
N003	Bimtakothi-3	R	4	1	3
N304	Bimtakothi-4	R	-	-	-
N604	Bimtakothi-5	S	-	-	-
N619	Bimtakothi-9	R	4	1	3
N019	Bimtakothi-10	R	4	1	3
N320	Bimtakothi-11	S	-	-	-
N620	Bimtakothi-12	R	-	-	-
N020	Bimtakothi-13	R	4	1	3
N033	Chame-2	S	4	3	3
N334	Chame-3	R	4	2	3
N045	Chame-8	R	4	3	3
N348	Chame-9	R	4	1	3
N048	Chame-11	R	4	1	3
N349	Chame-12	R	4	2	3
N649	Chame-13	S	4	3	3

Table 20 Cont.

OU No.	Landraces	Reaction to			
		BaMMV	Powdery mildew		Leaf rust
			Isolate 217	Isolate 178	Race J 80
N049	Chame-14	R	4	2	3
N047	Dhumpu-2	R	4	0	3
N650	Ghara-1	R	-	-	-
N050	Ghara-2	R	-	-	-
N308	Gho-1	S	-	-	-
N608	Gho-2	S	-	-	-
N008	Gho-3	S	4	1	3
N373	Jomson-1	S	4	1	3
N673	Jomson-2	R	-	-	-
N358	Kagbeni-3	R	-	-	-
N058	Kagbeni-5	S	4	2	3
N023	Lih Dhanra Gal	R	-	-	-
N084	N-6	S	1	0	3
N086	N-12	S	1	0	3
N387	Naked-304	R	3	1	3
N375	Nepal-1	R	-	-	-
N376	Nepal-4	S	-	-	-
N676	Nepal-5	S	-	-	-
N076	Neapl-6	S	-	-	-
N677	Nepal-7	S	-	-	-
N340	Ngyak-1	S	4	1.5	3
N640	Ngyak-2	S	4	2	3
N040	Ngyak-3	S	-	-	-
N341	Ngyak-4	R	-	-	-
N614	Tilman camp-7	R	4	1	3
N343	Ngyak-10	R	0.5	0	3
N643	Ngyak-11	R	4	1	3
N043	Ngyak-12	S	-	-	-
N351	Phalatey	S	4	2	3
N682	Philem-1	S	4	3	3
N082	Philem-2	S	4	0	3
N382	Philem-3	S	-	-	-
N323	Pisang-4	R	4	2	3
N623	Pisang-5	-	2.5	2	3
N346	Pisang-6	S	3	0.5	3
N646	Pisang-7	S	0.5	1	3
N652	Pisang-8	R	-	-	-
N052	Pisang-9	S	3	0.5	3
N344	Pork-1	S	4	3	3

Table 20 Cont.

OU No.	Landraces	Reaction to			
		BaMMV	Powdery mildew		Leaf rust
			Isolate 217	Isolate 178	Race J 80
N644	Pork-2	S	3	1.5	3
N005	Sama-1	S	3	3	3
N306	Sama-2	S	3	3	3
N606	Sama-3	S	3	4	3
N006	Sama-4	S	4	4	3
N307	Sama-5	-	4	4	3
N607	Sama-6	S	4	3	3
N007	Sama-7	-	4	4	3
N324	Sama-8	R	-	-	-
N624	Sama-9	R	1	2.5	3
N024	Sama-10	-	1.5	2	3
N353	Sikha-1	R	3	3	3
N653	Sikha-2	R	3	1.5	3
N354	Sikha-4	R	4	2	3
N654	Sikha-5	R	0	2	3
N054	Sikha-6	R	3	3	3
N355	Sikha-7	R	3	3	3
N655	Sikha-8	R	-	-	-
N077	Sipche-1	-	4	4	3
N377	Sipche-2	R	-	-	-
N678	Sipche-3	R	-	-	-
N078	Sipche-4	R	4	4	3
N679	Sipche-6	R	3	2	3
N079	Sipche-7	R	4	3	3
N379	Sipche-8	-	4	4	3
N680	Sipche-9	R	4	4	3
N080	Sipche-10	-	4	2	3
N380	Sipche-11	R	3	2	3
N681	Sipche-12	R	-	-	-
N081	Sipche-13	-	4	3	3
N017	Thangja-1	R	4	4	3
N318	Thangja-2	R	4	4	3
N618	Thangja-3	R	4	4	3
N659	Thomje-2	S	3	4	3
N059	Thomje-3	S	4	4	3
N360	Thomje-4	S	-	-	-
N660	Thomje-5	-	4	4	3
N060	Thomje-6	S	-	-	-
N361	Thomje-7	S	4	4	3

Table 20 Cont.

OU No.	Landraces	Reaction to			
		BaMMV	Powdery mildew		Leaf rust
			Isolate 217	Isolate 178	Race J 80
N601	Thonje-2	-	4	4	3
N001	Thonje-3	R	4	3	3
N302	Thonje-4	R	-	-	-
N602	Thonje-5	R	4	3	3
N002	Thonje-6	R	4	3	3
N035	Thonje-16	R	4	4	3
N336	Thonje-17	-	4	4	3
N636	Thonje-18	R	4	2	3
N036	Thonje-19	R	4	3	3
N337	Thonje-20	-	4	4	3
N637	Thonje-21	R	4	2	3
N037	Thonje-22	-	4	4	3
N009	Tilman camp-1	R	4	-	3
N014	Tilman camp-8	R	4	1	3
N645	Tsumje-1	S	-	-	-
N672	Tsumje-2	S	-	-	-
N671	Ulleri-21	S	-	-	-

BaMMV: R, Resistant; S, Susceptible

Powdery mildew and leaf rust reactions averaged on 10 and 20 test plants, respectively

Powdery mildew/leaf rust: 0, 1, 2 (Resistant); 3, 4 (Susceptible)

–, not tested

3.4 Genetic studies on BaMMV resistance of Nepalese hulless barley landraces

3.4.1 Diversity of BaMMV resistance genes

Allelism tests were performed between the selected BaMMV resistant landraces to get information whether different genes conferring resistance to BaMMV are present in Nepalese hulless barley germplasm. Eight F_2 progenies derived from 'resistant x resistant' crosses were evaluated for reaction to BaMMV (Table 21). Out of this, five progenies showed a uniform resistance reaction indicating that the cross parents carry allelic genes conferring resistance to BaMMV. However, the progenies of two crosses, i.e., Sipche-2 x Sikha-7 and Annapurna BC-1 x Ngyak-4, segregated into resistant and susceptible types. This is indicative of a non-allelic relationship or different genes for BaMMV resistance in the cross parents. Two suspected

susceptible plants were detected within the progeny of Thangja-2 x Chame-12; therefore, further tests are needed to get reliable information on allelism.

Table 21 Allelism test between the BaMMV resistant Nepalese hulless barley landraces

Crosses	F_2 progeny test for BaMMV
Annapurna BC-1 x Pisang-8	uniform resistant
Annapurna BC-1 x Pisang-4	uniform resistant
Thangja-2 x Chame-12	unclear segregation
Pisang-4 x Pisang-8	uniform resistant
Sipche-2 x Sikha-7	segregating[a]
Annapurna BC-2 x Pisang-4	uniform resistant
Annapurna BC-1 x Ngyak-4	segregating[b]
Sipche-2 x Tilman-1	uniform resistant

a, Nos. of plant tested=28; Resistant 19, Susceptible 9

b, Nos. of plant tested=25; Resistant 14, Susceptible 11

3.4.2 Identification of genes conferring BaMMV resistance in Nepalese hulless barley landraces

The F_1 progenies derived from the crosses between BaMMV resistant Nepalese landraces and German or exotic cultivars carrying known genes conferring resistance to BaMMV were evaluated for reaction to BaMMV. The results of the disease test are summarized in Table 22. Because of an overall low infection rate (infection rate of susceptible control 60-70 %); it is possible that some of the plants escaped infection and therefore, the F_1 progenies showed two classes of disease reaction, i.e., all resistant, and resistant and susceptible types. Therefore, detection of a susceptible F_1 plant indicates different genes conferring resistance to BaMMV, whereas complete resistance reaction indicates identical or allelic recessive genes in the cross parents.

With the present results, it is likely that Bimtakothi-10, Pisang-4, Sipche-2, Chame-12, Jomson-2 and Ghara-2 carry *rym4/rym5*. Similarly, Annapurna BC-1, Thonje-5, Sama-8 and Chame-8 carry *rym4/rym5* with an additional gene *rym9* or *rym12*. It is important to note here that some of the landraces positive for *Rym4/Rym5* locus were also positive for *Rym2*, e.g., Annapurna BC-1, Bimtakothi-10 and Sama-8. However, in previous studies it was shown that the carrier *Rym2*, i.e., Mihori Hadaka 3 also

carries *rym4* (Götz and Friedt 1993). The landrace Thangja 2 was found positive for *rym12* and *rym15*.

It was revealed that two landraces, namely Tilman Camp-1 and Sikha-7, carry BaMMV resistance genes different from *rym4/rym5*. Moreover, it is confirmed with the allelism test (Sikha-7 *vs* Sipche-2) that Sikha-7 does not carry *rym4/rym5* because Sipche-5 which was shown to be non-allelic to Sikha-7 carrying *rym4/rym5* (Tables 21 & 22). All the landraces that were crossed with Ishukushirazu, a cultivar carrying *rym3*, had susceptible progeny. In fact, *rym3* is not effective against BaMMV and therefore, a non-allelic relationship is expected between Ishukushirazu and the landraces resistant to BaMMV.

Table 22 Allelism tests between the BaMMV resistant Nepalese landraces and cultivars carrying known genes conferring resistance to BaMMV. The (+) sign indicates for allelic relationship and (-) indicates for non-allelic relationship

Landraces	Genes for allelism test									Allelic genes
	Rym2	*rym3*	*rym4*	*rym5*	*rym8*	*rym9*	*rym11*	*rym12*	*rym15*	
AnnapurnaBC-1	+	-	+	+	-	-	-	+	-	*rym4/rym5, rym12*
TilmanCamp-1		-	-	-	-	-		-	-	?
Bimtakothi-10	+		+	-	-	-	-		-	*rym2, rym4*
Thonje-5		-	+	+	-	+	-	-	-	*rym4/rym5, rym9*
Pisang-4	+ (?)		+	-	-	-		-	-	*rym4*
Pisang-8				-	-	-	-		-	?
Thangja-2	-	-		-	-	-	-	+	+	*rym12, rym15*
Sama-8	+	-	+	+	-	+	-	-	-	*rym4/rym5, rym9*
Sipche-2	-	-	+	+	-	-	-	-	-	*rym4/rym5*
Chame-8			+		-		-	+		*rym4, rym12*
Chame-12		-	+	+	-	-	-	-	-	*rym4/rym5*
Jomson-2			+	-	-	-	-		-	*rym4*
Ghara-2				+	-	-	-	-		*rym5*
Sikha-7	-	-	-	-	+ (?)	-	-			?

4. Discussion

4.1 Genetic diversity and population differentiation of Nepalese hulless barley landraces

The panel of SSRs used to assess the molecular diversity of Nepalese hulless barley proved to be highly informative (Table 12). The polymorphism information content (PIC) of the SSRs, selected from the set of SSRs proposed by Macaulay et al. (2001) for genotyping in barley, differed in the present study from the results reported by Macaulay et al. (2001). For example, SSRs Bmac0032, Bmac0113, Bmag0223, Bmac0273 and Bmac0156 are found to be highly informative on the set of Nepalese hulless barley landraces revealing a PIC value of >0.80, while e.g., Bmac0273 had a low PIC value (0.59) in the study of Macaulay et al. (2001). In contrast to this, SSRs WMC1E8, Bmag0013, Bmac0040, Bmac0316 and Bmac0218 were less informative in the present study. The mean PIC value of 41 polymorphic loci (0.50) is comparable to that of Hamza et al. (2004, PIC=0.45) estimated on 26 Tunisian winter barley accessions and cultivars with 17 SSRs. The PIC of an SSR marker, which is also defined as its capacity to discriminate genotypes, largely depends on the allelic diversity. In this respect, a correlation coefficient of r=0.76 between the PIC-values and the number of alleles detected was estimated. A strong positive correlation (r=0.624) between the gene diversity at an SSR locus (~PIC) and the number of alleles detected is also reported by Yu et al. (2003).

The genetic diversity index (DI) on the set of 107 landraces is estimated at DI=0.536. Russell et al. (2003) reported a DI=0.652 on a set of 125 barley lines collected from various regions of Syria and Jordan assessed with 20 SSRs of which 15 are common to the present experiment. Therefore, it may be concluded that a DI=0.536 on 107 hulless Nepalese barley landraces is moderate to high indicating that the highlands of Nepal are rich in barley diversity. This is in agreement with the findings of Witcombe and Murphy (1986), or Murphy and Witcombe (1986) based on the morphological variation and with results by Konishi and Matsuura (1991) based on isozyme diversity.

The landraces studied are divided into different groups using genetic similarity (GS) based UPGMA clustering and a Bayesian model based (MB) structure analysis (Pritchard et al. 2000). The MB method is widely used in population structure analysis for association studies in human and animal genetics. Recently, the method

that was developed by Pritchard et al. (2000) and provided as the software program STRUCTURE, is frequently being used for population structure analysis or to define genetically distinct groups in crop plants (Remington et al. 2001; Liu et al. 2003; Jain et al. 2004; Lu et al. 2005; Stich et al. 2005). The structure analysis revealed 9 groups of Nepalese hulless barley landraces out of which 8 distinct populations are identified (Figure 9). Using a similar approach, Remington et al. (2001) and Liu et al. (2003) were able to differentiate a large set of maize inbred lines into genetically distinct groups with high pedigree conformity.

The seven populations out of eight identified are localized in the map and represent specific geographic regions (Figure 4). The landraces named as 'Nepal' along with the landraces from Ghara constituted a population (MB group 2) which however could not be located in the map because the geographic origin of landraces named 'Nepal' was not known. All the landraces of Bimtakothi and many from Chame conferred a highly mixed ancestry (MB group 8). It is worthwhile to note that many landraces from Chame had an ancestry composition similar to the landraces of Bimtakothi and comprised a high proportion of mixed ancestry corresponding to Thonje origin (MB group 7). However, Chame-9, Chame-14 and Chame-8 were found with 98 %, 80 % and 61 % inferred ancestry origin to Jomson (MB group 5), respectively. Unlike the majority of the landraces of Bimtakothi, the landraces Bimtakothi-3, Bimtakothi-9 and Bimtakothi-11 showed a high conformity to the origin having >75 % inferred ancestry corresponding to Bimtakothi (MB group 8). These accessions however, shared a significant proportion of mixed ancestry of Nepal origin (MB group 2)

The complex genetic makeup of the landraces of Chame and Bimtakothi detected with the structure analysis can also be seen in UPGMA clustering as the landraces are widely dispersed in the dendrogram (Figure 8). The landraces with an inferred ancestry corresponding to a different origin, for example, Chame-9 and Thonje-21 found in Jomson (MB group 5), represent freshly introduced populations in the region that can result from seed exchange among the farmers.

Comparing GS based UPGMA clusters and the MB groups, the UPGMA clusters are in accordance to the origin only among the landraces sharing high genetic similarity, and the clusters rarely represent geographic groups. On the other hand, the MB approach was able to cluster landraces representing most appropriate geographic

groups that can be explained by the information available on landrace origin. Jain et al. (2004) reported highly comparable results of GS based UPGMA clustering and MB structure analysis. Similarly, Lu et al. (2005) found both approaches equally effective to define genetically distinct groups of 145 US rice cultivars, however, concluded that UPGMA clusters have a greater conformity with pedigree data. In this study, a difference existed between GS groups and MB groups, although results are comparable to a large extent. Based on the present results, it can be suggested that structure analysis can define more informative groups than GS based UPGMA cluster analysis when genotypes are of complex origin or the pedigree is not known, e.g., admixture populations and/or gene bank accessions.

The overall DI of 7 populations (64 landraces) and that of the whole sample (107 accessions) is estimated at the same level, 0.539 and 0.536, respectively. Likewise, the number of polymorphic SSRs detected among the 7 populations is at 97.6% of the whole sample even after the population size was reduced to 59.81%. This indicates that structure analysis was quite effective to define genetically distinct populations among the 107 landraces. The DI estimations for each of the 7 populations varied, and populations from the Marshyangdi and the BudhiGandaki region in the East are more diverse compared to those of the KaliGandaki region in the West (Table14).

The highest genetic diversity existed in the Pisang population (DI=0.559) which is larger than that estimated for the whole sample or within the sub-set of 64 landraces. This population consisted of five landraces of Pisang origin and the landraces are highly consistent to the origin having <1.00% mixed ancestry of any other origin (Table 13). Furthermore, considerable numbers of specific alleles were detected within the Pisang population which ranked second after Thonje (Table 14). Geographically, Pisang is fairly isolated and represents the uppermost basin of the river Marshyangdi (Figure 4). The region can be considered a hot spot of hulless barley diversity. Schoen and Brown (1991) have emphasized the existence of such hot spots of genetic variation in self pollinated crops which are of high relevance to the conservation of genetic resources.

The population differentiation parameters estimated, θ=0.433 and R_{ST}=0.445, indicated a high level of differentiation, and over 40% of the total genetic variation resided among the 7 populations. The R_{ST} value is estimated slightly higher than the

θ-value. Similar results are reported by Zhou et al. (2003) for different rice populations (θ=0.491; R_{ST}=0.519). The comparable values of the θ and R_{ST} in this study suggest SSRs do not fit strictly to either of the mutation models (SMM or IAM).

The population-pairwise differentiation test revealed that not all of the 7 populations are significantly different from each other (Table 15). Indicated by the estimated high DI value and the fact of not being differentiated from all the populations except Thonje, it may be concluded that landraces from the Pisang region possess a broad genetic base and can be considered as a founding population in the highlands of central Nepal. However, highly differentiated landraces found in Thonje which is geographically close to Pisang makes it difficult to explain whether the Thonje population is a descendent of Pisang or evolved independently. The highly differentiated landraces and many conserved alleles found in Pisang and Thonje affirmed the upper valley of river Marshyangdi as the origin of hulless barley diversity within the Himalayas-range in central Nepal.

When the genetic relationship among the populations is compared to geographic distances, striking results are found. For example, the population Ngyak is found genetically closer to Thonje and Pisang than to Sipche which is geographically closer to Ngyak. Similarly, the populations Sipche and Sikha are genetically close, however, are geographically most distant. These findings are also verified by the test of isolation by distance hypothesis resulting in a non-significant correlation (r=0.224, p>0.05) between pairwise θ and the geographic distance. However, the influence of geographic distance on the genetic relationship can be deduced from the mean inferred ancestry (%) of the MB groups (Table 13). Most of the groups comprised the largest proportion of mixed ancestry from the nearest group indicating adjacent populations shared common parentage to some extent.

The patterns of diversity and genetic relationship among the populations are largely related to the altitude that varies sharply from the South to the North creating a range of agro-ecological environments, e.g., warm temperate climate with high monsoon rain in the South (Sikha) and cool temperate or sub-alpine climate affected by the rain shadow of the Himalayas in the North (Upper valleys of KaliGandaki and Marshyangdi). The distribution of hulless barley is more frequent in higher altitudes towards the North (Baniya et al 1997), and its value as the sole food crop increases with the increasing altitude where other cereals can not be grown successfully

(Sharma et al. 1994). In this study, most diverse landraces were found at the highest altitude in the North, i.e., Pisang (~3500 m). A positive correlation between the magnitude of genetic diversity and altitude of collection site has been reported by Konishi et al. (1986) (cited in Konishi et al. 1993) in naked barley populations derived from the eastern Himalayas of Nepal.

The populations from Thonje, Ngyak and Sipche (Altitude ≤2800 m) showed a comparable level of diversity, however, less than that estimated for Pisang (Table 14). The patterns of diversity detected on populations from the upper basins of Marshyangdi, BudhiGandaki and the East of BudhiGandaki are in accordance with the trends of hulless barley distribution in relation to altitude. However, the diversity estimated on populations derived from the KaliGandaki region in the West did not concur with latitudinal variation, i.e., populations from the North (Jomson, altitude ~3000 m) and the South (Sikha, altitude ~2000 m sea level) revealed the same level of diversity, which is significantly lower than in the populations from the East (Table 14). The less diverse hulless barley populations observed in the KaliGandaki valley may be due to a high preference for hulled types in this region. The genetic relatedness among the populations also reflected a North-South differentiation pattern with some exceptions (Figure 10). For example, landraces from the upper basin of KaliGandaki are genetically close to the landraces from the upper basins of Marshyangdi and BudhiGandaki, and are distinct from those originated from the South (Sikha). However, a closer association between the populations Sipche and Sikha, one derived from a higher altitude in the East and the other from a lower altitude in the West, respectively, needs some further explanation. Factors other than agronomic and eco-geographic, e.g., historical and /or ethnicity, may have a role which must be considered in future studies. Moreover, information on the origin of the landraces named ‘Nepal’ would help to further elucidate the genetic and eco-geographic relationship of the barley populations.

The Himalayas are well known to harbor a tremendous diversity in cultivated barley and therefore are considered a region of domesticated barley diversification (Badr et al. 2000; Li et al. 2004). The present study also revealed considerable genetic diversity and highly complex genetic structure of the Himalayan barley populations supporting this statement. Genetic differentiation results from the joint effects of mutation, migration, selection and drift, which in turn must operate within the

historical and biological context of each plant species. In crop plants, human selection plays a major role in shaping population structure. However, in case of landrace populations which are found in more natural environments, natural selection in response to environmental heterogeneity (biotic and abiotic) is the major cause of population differentiation (Linhart and Grant 1996). The diverse and highly differentiated barley landraces found in the Himalayas can be primarily attributed to the vast eco-geographical diversity prevailing in the region. Furthermore, frequent seed exchange among the farming communities seen in the highland agriculture definitely accelerates the process of diversification and contributes to complicate population structures.

4.2 Genetic relationship of Nepalese hulless barley with East Asian and Western barley cultivars

The barley landraces from the highland of Nepal, Himalayas, are widely represented in the international gene banks and therefore, are an important genetic resource for barley breeding. These landraces are frequently used by barley researchers in genetic studies (Liu et al. 1999; Treuren and Hintum 2001; Taketa et al. 2004) or search for economically important traits, e.g., disease resistance (Mueller and Enneking 2003). To provide a basis for more effective utilization of the Himalayan barley genetic resource, the genetic relatedness of Nepalese hulless barley landraces to main stream barley cultivars derived from Europe and East Asia was analyzed.

The 30 mapped SSRs analyzed on 161 diverse barley genotypes demonstrated a wide range of allelic variation and diversity (Table 16). The mean number of alleles per locus as an indicator of overall allelic richness was high (7.9 alleles/locus). The mean gene diversity (Nei 1978) which is also termed as diversity index (DI) of the sample was estimated at DI=0.606. At individual loci, Bmac0032 (1H), Bmag0007 (7H) and Bmag0223 (5H) showed the highest level of diversity (> 0.80). These SSRs, particularly, Bmac0032 and Bmag0007 possessed wide range of alleles. For example, in total 24 and 21 alleles were detected for Bmac0032 and Bmag0007, respectively. Saghai Maroof et al. (1994) demonstrated that extraordinary microsatellite allele diversity exists in barley. They detected as many as 37 alleles at a single locus (HVM4) on a large set of cultivated and wild forms of barleys. More

recently, Malysheva-Otto et al. (2006) reported the highest number of alleles at Bmac0032 locus (n=33) on a world collection of 953 cultivated barley accessions. In the present study, some of the SSRs, e.g., HvHVA1 (1H) and HvLOX (5H) are characterized with extremely low level of allele diversity (Table 16).

The analysis of unique alleles of the SSRs within different groups of barleys revealed that nearly half of the total alleles correspond to a specific origin or group of barley. The highest number of unique alleles was found in Nepalese landraces (n=36). Similarly, a considerable numbers of alleles were found to be specific to German set of barley cultivars (n=21). Although the numbers of unique alleles detected between these two groups of barleys are not directly comparable due to the large difference in sample size, the results, however can be interpreted in relative terms. For example, 21 unique alleles detected on the set of 35 German cultivars can be considered relatively high compared to 36 detected on the set of 107 Nepalese landraces.

The high allelic diversity of German cultivars is also reflected in estimated mean gene diversity of the sample which is higher than that of Nepalese landraces (Table 16). In this respect, Koebner et al. (2003), in a retrospective analysis of diversity in the UK barley (1925 to 1995), demonstrated that systematic plant breeding does not inevitably lead to a reduction in genetic diversity. Khlestkina et al. (2004) demonstrated similar results in cultivated wheat (*Triticum aestivum* L.) samples collected over an interval of 40−50 years in four comparable geographical regions of Europe and Asia. It was found that an allele flow took place during the adaptation of traditional agriculture to modern systems, whereas the level of genetic diversity was not significantly influenced.

In contrast to this, Russell et al. (2000) reported a reduction in genetic diversity of modern barley cultivars over time compared to that of the foundation genotypes. However, Ordon et al. (2005) reported considerable increase in overall genetic diversity in German two-row barley in the last 50 years due to extensive breeding efforts. The large number of unique alleles and high level of diversity found in the German set of barley cultivars can be justified by the fact that it comprised of modern as well as old cultivars covering a wide range of time period (1891 to 1999). Relative to the sample size, the number of group specific alleles detected within the East Asian, East European and Canadian barley cultivars are also high clearly indicating for the geographic differentiation of these barleys (Table 17). This is in agreement

with the findings of Malysheva-Otto et al. (2006), who analyzed the population structure in a worldwide survey of cultivated barley, and reported highly differentiated geographic populations derived from Asia, Near East, Europe, Africa and America.

The patterns of allelic richness and diversity estimated at each SSR locus separately on Nepalese landraces (NL) and German cultivars (GC) showed differences in these two groups (Table 16). Almost 90 % of the rare alleles (freq. <0.05) and over 50 % of the common alleles (freq. >0.05) analyzed on the whole sample (NL+GC) were uniquely found in either of the two groups (NL or GC) (Table 17). This shows the high level of genetic differentiation between the Nepalese hulless barley and German hulled cultivars. This is in conformity with the results of Terzi et al. (2001), in which it is shown that Nepalese landraces are genetically different from Western barleys.

The SSRs diversity at specific genomic regions corresponding to the 7 barley chromosomes was analyzed. While on the whole sample (NL+GC), the mean gene diversity (Nei 1978; unbiased) for all the 7 chromosomes were highly comparable, a clear difference was observed for 2H and 6H when separately estimated on Nepalese landraces and German cultivars (Table 18). On the set of German cultivars the diversity detected at 2H was considerably low, whereas Nepalese landraces showed the similar pattern of suppressed diversity at 6H. Furthermore, the same trend of reduced diversity of SSRs at 6H (measured as PIC) on Nepalese landraces was detected when it was compared to the Western cultivars and accessions analyzed by Macaulay et al. (2001) (Table 12).

The results showed evolutionary differences at microsatellite loci corresponding to 2H and 6H genomic regions between the Himalayan hulless barley and German hulled cultivars. Backes et al. (2003) analyzed the RFLP diversity within and between major groups of barley in Europe, and demonstrated that diversity patterns detected on genomic segments (chromosomes) can be related to the agronomic, adaptation or historical aspects of the barley cultivars. For example, the chromosomal distribution of RFLP diversity analyzed between the groups of barley with different row-type and growth habits (two-rowed and six-rowed spring type; two-rowed and six-rowed winter type), revealed that differences in diversity at 4H and 5H may be related to adaptation to spring/winter growing conditions. Similarly, the differences in diversity at 1H and 3H were found to be related with the ear-type differentiation, and six-rowed winter type *vs* other groups of barley, respectively. In this respect, the differences in

SSRs diversity detected at 2H and 6H genomic regions on Nepalese landraces and German cultivars may be related to Oriental−Occidental differentiation of cultivated barley.

The UPGMA clustering was highly effective to group barley cultivars/accessions according to their origin. The highest order clusters representing two broad groups clearly differentiated the eastern and western world barleys (Figure 13). These results are in accordance with the report of Ordon et al. (1997), who found a clear differentiation between East Asian and German cultivars. The two *H. spontaneum* accessions derived from Israel can be seen distinct from the cultivated forms of barley in the dendrogram, however, had a closer relationship with the Western cultivars than with the barleys derived from the East. This can be explained by considering that cultivated barley evolved from the two-rowed wild barley (*H. spontaneum*) in the Fertile Crescent (Badr et al. 2000), and therefore, it can be expected that Western or European barleys have a closer genetic relationship with wild barley from Israel than to Himalayan or East Asian barleys. However, Strelchenko et al. (1999) reported that some of the *H. spontaneum* accessions have a closer relationship with Oriental genotypes of cultivated barley and suggested that the broad clustering into Oriental and Occidental types may reflect different source of wild barley germplasm being the reason for two genetic groups of cultivated barley.

Within the barleys from the East, Nepalese hulless barley landraces were highly consistent in clustering, and were distinct from the East Asian hulled cultivars. Moreover, Canadian naked cultivars were clearly distinguished from the Nepalese naked barley and also from the European hulled cultivars. The closer genetic relationship between the Japanese malting barley varieties, Misato Golden and Resistance Ym No.1, and European barley cultivars (Figure 13) can be explained by the fact that these varieties possibly obtained their malting quality from European cultivars (Ordon et al. 1997). The present study showed a clear genetic differentiation of cultivated barley from East Asia, Himalayas, Europe and North America. In addition to the geographic differentiation, the barley samples analyzed here, particularly the Nepalese landraces and the German cultivars represent a contrasting genetic background. For example, Nepalese landraces are local populations adapted to stress environments and are considerably tailored by the diverse natural selection forces. In contrast to this, German cultivars are the product of intensive selection and

breeding to fit the specific cropping environments. The genomic contrast between these two sets of barley can be utilized in genetic mapping or for the localization of complex, however, economically important traits.

4.3 Disease reaction of Nepalese hulless barley landraces

Nepalese hulless barley landraces were evaluated for resistance to Barley mild mosaic virus (BaMMV), powdery mildew (*B. graminis* f. sp. *hordei*) and leaf rust (*P. hordei*). Artificially induced infection and subsequent DAS-ELISA assessment showed that many of the Nepalese hulless barley landraces are resistant to BaMMV (Table 20). A high frequency of resistant landraces was found in some localities, e.g., Sikha, Sipche, Thonje, Chame and Bimtakothi. However, there was no specific pattern of geographic distribution of the BaMMV resistance.

The allelism test between the resistant landraces revealed that diversity exists for BaMMV resistance in Nepalese hulless barley germplasm (Table 21). Preliminary genetic studies indicated that BaMMV resistance in Nepalese hulless barley is mainly due to the R*ym4/Rym5* locus, however, some of the landraces were positive for *rym9* and *rym12* (Table 22). Furthermore, it is likely that Annapurna-1, Thonje-5, Thangja 2, Sama-8 and Chame-8 carry more than one resistance gene. Barley genotypes with multiple resistance genes for BaMMV/BaYMV have been reported by Ordon and Friedt (1993), or Götz and Friedt (1993). Due to the fact that the F_1 progeny test was based on a small number of plants and the overall infection rate was low, some of the results have to be confirmed by F_2 progeny analysis.

The hulless barley landraces reacted differently to powdery mildew (*B. graminis* f. sp. *hordei*) and leaf rust (*P. hordei*) diseases, respectively (Table 20). A large number of landraces were resistant to powdery mildew Race−D40/4, however, very few landraces showed resistance reaction to Race−D12/12. Most of these landraces are reported to be susceptible to Japanese races of powdery mildew (Catalogue of Barley Germplasm Preserved in Okayama Univ. 1983). The race specific resistance pattern indicated that Nepalese hulless barley germplasm carry mainly, We, U2, / St, U2 / Ly, We, Kw, La / Ha / Mlp / Mla22 / Mla27 types of powdery mildew resistance.

Regarding leaf rust Race J 80, none of the landraces showed a resistance reaction. The present results are in conformity with the report of Baniya et al. (1997) that Nepalese barley landraces are highly susceptible to leaf rust. The low frequency of

fungal disease resistance, e.g., mildew and rusts, in Nepalese hulless barley germplasm may be due to the lack of disease pressure in the highlands of the Himalayas.

5. Summary

The Himalayas are known as a region of domesticated barley diversification. Barley landraces from the Himalayas, particularly from the highlands of Nepal, share a significant part of the world barley germplasm resources. Due to the fact that hulless barley is widely grown in the highlands of Nepal from the East to the West along the Himalayas, it is frequently represented in Himalayan barley collections and can be considered as an important genetic resource. The Nepalese hulless barley landraces are frequently being used by the barley researchers; however, a more effective utilization of this genetic resource is hindered due to the lack of detailed information on genetic diversity and population differentiation. Furthermore, the extent of genetic relatedness of Nepalese hulless barley to the mainstream barley cultivars is not known.

In the present investigation, a large set of hulless barley (*Hordeum vulgare* L. subsp. *vulgare*) landraces originally collected from the highlands of Nepal along the Annapurna and Manaslu Himalaya-range were analyzed for genetic relatedness and population differentiation using simple sequence repeats (SSRs). The 44 genome-covering barley SSRs revealed a high level of genetic diversity among the landraces (diversity index, DI=0.536). The genetic similarity based UPGMA clustering and Bayesian model based structure analysis revealed a complex genetic structure of the landraces. Eight genetically distinct populations were identified, of which 7 were further studied for diversity and differentiation.

The populations were fairly differentiated (θ=0.433, R_{ST}=0.445) accounting for >40% of the genetic variation among the populations. The pairwise population differentiation test confirmed that many of the geographic populations significantly differ from each other but that the differentiation is independent of the geographic distance (r=0.224, p>0.05). The genetic diversity estimated for all and each population separately revealed a hot spot of genetic diversity at Pisang (DI=0.559). The highlands of central Nepal, particularly valleys along the upper basin of the river Marshyangdi (Pisang and Thonje) are rich in hulless barley diversity, and landraces in this region are highly differentiated. The upper basin of Marshyangdi can be considered as origin of hulless barley diversity within the Himalayas range in central Nepal.

The Nepalese hulless barley landraces are found genetically different from the Western and East Asian hulled as well as hulless cultivars. A detail analysis of frequency and distribution of SSR alleles between the Nepalese landraces and German hulled cultivars revealed several unique alleles. Furthermore, a significant difference in SSRs diversity estimated at genomic regions corresponding to chromosomes 2H and 6H was found between these two sets of barleys.

The landraces showed resistance reaction to Barley mild mosaic virus (BaMMV) and powdery mildew (*Blumeria graminis* f. sp. *hordei,* Race D 12/12 and Race 178). However, a high degree of susceptibility was observed for leaf rust (*Puccinia hordei*, Race J 80). The preliminary genetic analysis indicated that diversity exists for genes conferring resistance to BaMMV and powdery mildew in Nepalese hulless barley germplasm.

The Nepalese hulless barley can be considered as an important genetic resource for mainstream barley breeding, particularly for spring types. This will broaden the genetic base of the present barley gene pool and provide novel alleles to the mainstream barley cultivars. The hulless barley populations can be used in genetic mapping to complement existing maps or for association studies to localize complex traits of agronomic importance. The small, isolated local populations like that have been described for Nepalese hulless barley germplasm can be more effective for an association study than large cosmopolitan populations.

6. Zusammenfassung

Landrassen der Gerste aus der Hochlandregion des Himalayas, insbesondere den Hochebenen Nepals, repräsentieren einen bedeutenden Teil der weltweiten genetischen Diversität der Gerste. Aufgrund des verstärkten Anbaus von Nacktgersten in dieser Region, stellen diese eine wichtige genetische Ressource für die Züchtung dar. Um das nepalesische Nacktgersten-Sortiment züchterisch effektiver zu nutzen, ist eine Charakterisierung der genetischen Diversität und differentiellen Beschreibung der Populationen notwendig. Darüber hinaus ist bisher wenig über die genetische Beziehung der nepalesischen Nacktgersten zu etablierten Gerstensorten bekannt.

Ein umfassendes Sortiment nacktsamiger Gersten-Landrassen (*Hordeum vulgare* L. subsp. *vulgare*) aus dem nepalesischen Hochland wurde hinsichtlich der genetischen Beziehungen und Populationsstrukturen mittels 44 genomabdeckender Mikrosatelliten-Marker (SSRs) analysiert. Dabei wurde eine hohe genetische Diversität innerhalb der Landrassen festgestellt (Diversitätsindex, DI=0.536). Untersuchungen zur Populationsstruktur mittels UPGMA-Clusteranalyse (basierend auf genetischer Ähnlichkeit) und Bayesian Modell-basierten Strukturanalyse ergaben ein komplexes Muster der Verwandtschaft zwischen den Landrassen, welche in distinkte geographische Populationen differenziert werden konnten (θ=0.433, R_{ST}=0.445). Obwohl mehr als 40% der genetischen Variation zwischen den Populationen vorliegt, ist diese unabhängig von der geographischen Distanz (r=0.224, p>0.05). Die genetische Diversität insgesamt sowie differenziert nach Populationen zeigt ein Maximum in der Population Pisang (DI=0.559). Insbesondere die Täler entlang des unteren Marshyangdi-Flusses (Pisang und Thonje) weisen eine hohe genetische Diversität und Differenzierung auf und können innerhalb der Himalaya-Region Zentral-Nepals als Genzentren unbespelzter Gerste betrachtet werden. Im Vergleich zu etablierten Sorten des Westens und Ost-Asiens sind nepalesische Nacktgersten genetisch deutlich differenziert und weisen spezifische SSR-Allele auf. Weiterhin konnte ein signifikanter Unterschied der allelischen Variation auf den Gersten-Chromosomen 2H und 6H festgestellt werden.

Innerhalb des nepalesischen Nacktgersten-Sortiments konnten Resistenzen gegenüber *Barley mild mosaic inducing virus* (BaMMV) und Echtem Mehltau (*Blumeria graminis* f. sp. *Hordei,* Race D 12/12 & Race 178) identifiziert werden,

während gegenüber Zwergrost (*Puccinia hordei*, Race J 80) eine hohe Anfälligkeit zu verzeichnen ist. Da die nepalesischen Nacktgersten ein hohes Maß an genetischer Diversität aufweisen, stellen sie eine potentielle Ressource und wertvolle Quelle neuer Allele für die Erweiterung der genetischen Diversität von Zuchtmaterial, insbesondere von Sommerformen der Gerste dar. Das analysierte Material ist weiterhin von Interesse im Rahmen genetischer Kartierungen, bspw. der detektierten Pilz- und Virus-Resistenzen, oder für Assoziationsstudien komplexer Merkmale mit agronomischer Bedeutung, wofür kleine, isolierte lokale Populationen wie die hier beschriebenen effektiver sind als große kosmopolitische Materialsets.

7. References

Åberg E (1940) The taxonomy and phylogeny of *Hordeum* L. Sect. *Cerealia* Ands. with special reference to Tibetan barleys. Symbolae Bot Upsalienses 4: 1-156

Abu Assar, AH, Uptmoor R, Abdelmula AA, Salih M, Ordon F, Friedt W (2005) Genetic variation in sorghum germplasm from Sudan, ICRISAT, and USA assessed by simple sequence repeats (SSRs). Crop Sci 45: 1636-1644

Assefa A, Labuschagne MT (2004) Phenotypic variation in barley (*Hordeum vulgare* L.) landraces from north Shewa in Ethiopia. Biodivers Conserv 13: 1441-1451

Backes G, Hatz B, Jahoor A, Fischbeck G (2003) RFLP diversity within and between major groups of barley in Europe. Plant Breeding 122: 291-299

Badr A, Müller K, Schäfer-Pregl R, El Rabey H, Effgen S, Ibrahim HH, Pozzi C, Rohde W, Salamini F (2000) On the origin and domestication history of barley (*Hordeum vulgare*). Mol Biol Evol 17: 499-510

Balloux F, Moulin NL (2002) The estimation of population differentiation with microsatellite markers. Mol Ecol 11: 155-165

Baniya BK, Dongol DMS, Riley KW (1997) Characterization of Nepalese barley germplasm. Rachis 16: 16-19

Bauer E, Weyen J, Schiemann A, Graner A, Ordon F (1997) Molecular mapping of novel resistance genes against barley mild mosaic virus (BaMMV). Theor Appl Genet 95: 1263-1269

Behall KM, Scholfield DJ, Hallfrisch J (2004) Diets containing barley significantly reduce lipids in mildly hypercholesterolemic men and women. Am J Clin Nutr 80: 1185-1193

Bennett MD, Smith LB (1976) Nuclear DNA amounts in angiosperms. Philosophical Transactions of the Royal Society (London) B Biological Sciences 274: 227-274

Bhatty RS (1999) The potential of hull-less barley. Cereal Chem 76: 589-599

Blattner FR, Mendez AGB (2001) RAPD data do not support a second center of barley domestication. Genet Resour Crop Evol 48: 13-19

Bothmer von R, Jacobsen N, Baden C, Jørgensen RB, Linde-Laursen I (1991) An ecogeographical study of the genus *Hordeum*, 2nd ed. IPGRI, Rome

Chabane K, Ablett GA, Cordeiro GM, Valkoun J, Henry RJ (2005) EST *versus* genomic derived microsatellite markers for genotyping wild and cultivated barley. Genetic Resour Crop Evol 52: 903-909

Chen JP, Adams MJ, Zhu FT, Wang ZQ, Chen J, Huang SZ, Zhang ZC (1996) Responses of foreign barley cultivars to barley yellow mosaic viruses at different sites in China. Plant Pathol 45: 1117-1125

Clark MJ, Adams AN (1977) Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J Gen Virol 34: 475-483

Dice LR (1945) Measures of the amount of ecologic association between species. Ecology 26: 297-302

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12: 13-15

FAOSTAT data (2006) http://www.faostat.fao.org/faostat, last assessed February 2006

Fischbeck G (2002) Contribution of barley to agriculture: A brief overview. In G A Slafer, JL Molina-Cano, R Savin, JL Araus and I Romagosa (eds) Barley Science: Recent Advances from Molecular Biology to Agronomy of Yield and Quality. Food Products Press, an imprint of the Haworth Press, Inc, Binghamton, NY pp 1-14

Friedt W (1983) Mechanical transmission of soil-borne barley yellow mosaic virus. Phytopathol Z 106: 16-22

Friedt W, Rasmussen M (2003) Modern European Barley Cultivars: Genetic Progress in Resistance, Quality and Yield. Paper presented at EUCARPIA cereal section meeting 2003, Italy

Friedt W, Werner K, Ordon F (2000) Genetic progress as reflected in highly successful and productive modern barley cultivars. Proceedings of the 8th International Barley Genetics Symposium 2000, Adelaide, Australia, Vol I, pp 271-279

Gill S, Vasanthan T, Ooraikul B, Rossnagel B (2002) Wheat bread quality as influenced by the substitution of waxy and regular barley flours in their native and extruded forms. J Cereal Sci 36: 219-237

Goodman SJ (1997) R_{ST} Calc: a collection of computer programs for calculating estimates of genetic differentiation from microsatellite data and determining their significance. Mol Ecol 6: 881-885

Götz R, Friedt W (1993) Resistance to the barley yellow mosaic virus complex – differential genotypic reactions and genetics of BaMMV-resistance of barley (*Hordeum vulgare* L.). Plant Breed 111: 125-131

Goudet J (2002) FSTAT version 2.9.3.2. A program to estimate and test gene diversities and fixation indices. Institute of Ecology, Lausanne, Switzerland. http://www.unil.ch/izea/softwares/fstat

Goudet J, Raymond M, Meeüs T, Rousset F (1996) Testing differentiation in diploid populations. Genetics 144: 1933-1940

Graner A, Bauer E (1993) RFLP mapping of the *ym4* virus resistance gene in barley. Theor Appl Genet 86: 689-693

Graner A, Bauer E, Kellermann A, Proeseler G, Wenzel G, Ordon F (1995) RFLP analysis of resistance to the barley yellow mosaic virus complex. Agronomie 15: 475-479

Graner A, Kota R, Perovic D, Potokina E, Prasad M, Scholz U, Stein N, Thiel T, Varshney RK, Zhang H (2004) Molecular mapping: shifting from the structural to the functional level. Proceedings of the 9th International Barley Genetics Symposium, 2004, Brno, Czech Republic pp 49-57

Graner A, Streng S, Kellermann A, Proeseler G, Schiemann A, Peterka H, Ordon F (1999b) Molecular mapping of genes conferring resistance to soil-borne viruses in barley. An approach to promote understanding of host-pathogen interactions. J Plant Dis Prot 106: 405-410.

Graner A, Streng S, Kellermann A, Schiemann A, Bauer E, Waugh R, Pellio B, Ordon F, (1999a) Molecular mapping of the *rym5* locus encoding resistance to different strains of the Barley Yellow Mosaic Virus Complex. Theor Appl Genet 98: 285-290

Grausgruber H, Bointner H, Tumpold R, Ruckenbauer P (2002) Genetic improvement of agronomic and qualitative traits of spring barley. Plant Breeding 121: 411-416

Hamza S, Hamida WB, Rebai A, Harrabi M (2004) SSR-based genetic diversity assessment among Tunisian winter barley and relationship with morphological traits. Euphytica 135: 107-118

Hariri D, Meyer M, Le Gouis J, Bahrman N, Fouchard M, Forget C, Andre A (2000) Characterization of BaYMV and BaMMV pathotypes in France. European J Plant Path 106: 365-372

Hariri D, Meyer M, Prud'homme H (2003) Characterization of a new barley mild mosaic virus pathotype in France. European J Plant Path 109: 921-928

Harlan JR, Zohary D (1966) Distribution of wild wheats and barley. Science 153: 1074-1080

Harwood WA, Bilham LJ, Travella S, Bourdon V, Salvo H, Harden J, Perry M, Snape JW (2004) Genetic transformation of Barley: improved technology, safety assessment and future potential. Proceedings of the 9th International Barley Genetics Symposium, 2004, Brno, Czech Republic pp 112-118

Huth W (1989) Ein weiterer Stamm des Barley Yellow Mosaic Virus in der Bundesrepublik Deutschland. Nachrichtenbl Deut Pflanzenschutzd 40: 49-55

Huth W, Adams MJ (1990) Barley Yellow Mosaic Virus (BaYMV) and BaYMV-M: Two different viruses. Intervirology 31: 38-42

Huth W, Lesemann DE (1978) Eine für die Bundesrepublik Deutschland neue Virose der Wintergerste. Nachrichtenbl Deut Pflanzenschutzd 36: 184-185

Iida Y, Ban T, Konishi T (1999) Linkage analysis of the *rym6* resistance gene to Japanese strain II of barley yellow mosaic virus (BaYMV-II) in barley. Barley Genet Newsl 29: 31-32

Iida Y, Konishi T (1994) Linkage analysis of a resistance gene to barley yellow mosaic virus strain II in two rowed barley. Breeding Science 44: 191-194

Ikata S, Kawai I (1940) Studies on wheat yellow mosaic disease. Noji Kairyo Shiryo 154: 1-123

Jahoor A, Fischbeck G (1987) Sources of resistance to powdery mildew in barley lines derived from *H. spontaneum* collected in Israel. Plant Breed 99: 274-281

Jain S, Jain RK, McCouch SR (2004) Genetic analysis of Indian aromatic and quality rice (*Oryza sativa* L.) germplasm using panels of fluorescently-labelled microsatellite markers. Theor Appl Genet 109: 965-977

Jakob SS, Meister A, Blattner FR (2004) The considerable genome size variation of *Hordeum* species (Poaceae) is linked to phylogeny, life forms, and speciation rates. Mol Biol Evol 21: 860-869

Kanyuka K, Druka A, Caldwell DG, Tymon A, Mccallum N, Waugh R, Adams MJ (2005) Evidence that the recessive bymovirus resistance locus *rym4* in barley corresponds to the eukaryotic translation initiation factor 4E gene. Mol Plant Path 6: 449-458

Kanyuka K, McGrann G, Alhudaib K, Hariri D, Adams MJ (2004) Biological and sequence analysis of a novel European isolate of Barley mild mosaic virus that overcomes the barley *rym5* resistance gene. Arch Virol 149: 1469-1480

Kays SE, Shimizu N, Barton FE, Ohtsubo K (2005) Near-Infrared transmission and reflectance spectroscopy for the determination of dietary fiber in barley cultivars. Crop Sci 45: 2307-2311

Khlestkina EK, Huang XQ, Quenum FJB, Chebotar S, Röder MS, Börner A (2004) Genetic diversity in cultivated plants − loss or stability? Theor Appl Genet 108: 1466-1472

Kikuchi S, Taketa S, Ichii M, Kawasaki S (2003) Efficient fine mapping of the naked caryopsis gene (*nud*) by HEGS (High Efficiency Genome Scanning)/AFLP in barley. Theor Appl Genet 108: 73-78

Knörzer KH (2000) 3000 years of agriculture in a valley of the High Himalayas. Veget Hist Archaeobot 9: 219-222

Koebner RMD, Donini P, Reeves JC, Cook RJ, Law JR (2003) Temporal flux in the morphological and molecular diversity of UK barley. Theor Appl Genet 106: 550-558

Konishi T, Ban T, Iida Y, Yoshimi R (1997) Genetic analysis of disease resistance to all strains of BaYMV in a Chinese barley landrace, Mokusekko 3. Theor Appl Genet 94: 871-877

Konishi T, Matsuura S (1991) Geographic differentiation in isozyme genotypes of Himalayan barley (*Hordeum vulgare*). Genome 34: 704-709

Konishi T, Yano Y, Fukushima Y (1993) Genetic variation in barley landraces from Bhutan. Genet Resour Crop Evol 40: 33-38

Konishi T, Ordon F, Furusho M (2002) Reactions of barley accessions carrying different *rym* genes to BaYMV and BaMMV in Japan and Germany. Barley Genet Newsl 32: 46-48

Kraakman ATW, Niks RE, Van den Berg PMMM, Stam P, Van Eeuwijk FA (2004) Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars. Genetics 168: 435-446

Le Gouis J, Devaux P, Werner K, Hariri D, Bahrman N, Beghin D, Ordon F (2004) *Rym*15 from the Japanese cultivar 'Chikurin Ibaraki 1' is a new Barley Mild Mosaic Virus (BaMMV) resistance gene mapped on chromosome 6H. Theor Appl Genet 108: 1521-1525

Lee KJ, Kashiwazaki S, Hibi T, So IY (1996) Properties and capsid protein gene sequence of a Korean isolate of barley mild mosaic virus. Annals of Phytopathological Society of Japan 62: 397-401

Levine MN, Cherewick WJ (1952) Studies on dwarf leaf rust of barley. US Department of Agriculture, Tech Bull 1056 p17

Li X, Xu C, Zhang Q (2004) Ribosomal DNA spacer-length polymorphisms in three samples of wild and cultivated barleys and their relevance to the origin of cultivated barley. Plant Breed 123: 30-34

Linhart YB, Grant MC (1996) Evolutionary significance of local genetic differentiation in plants. Annul Rev Ecol Syst 27: 237-277

Liu F, Bothmer R von, Salomon B (1999) Genetic diversity among East Asian accessions of the barley core collection as revealed by six isozyme loci. Theor Appl Genet 98: 1226-1233

Liu K, Goodman M, Muse S, Smith JS, Buckler Ed, Doebley J (2003) Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites. Genetics 165: 2117-2128

Lu H, Redus MA, Coburn JR, Rutger JN, McCouch SR, Tai TH (2005) Population structure and breeding patterns of 145 U.S. rice cultivars based on SSR Marker analysis. Crop Sci 45: 66-76

Macaulay M, Ramsay L, Powell W, Waugh R (2001) A representative, highly informative 'genotyping set' of barley SSRs. Theor Appl Genet 102: 801-809

Malysheva-Otto LV, Ganal MW, Röder MS (2006) Analysis of molecular diversity, population structure and linkage disequilibrium in a worldwide survey of cultivated barley germplasm (*Hordeum vulgare* L.). BMC Genetics 2006, doi: 10.1186/1471-2156-7-6

Manninen I, Schulman AH (1993) BARE-1, a copia-like retroelement in barley (*Hordeum vulgare* L.). Plant Mol Biol 22: 829-846

Mantel M (1967) The detection of disease clustering and generalized regression approach. Cancer Res 27: 209-220

McGrann GRD, Adams MJ (2004) Investigating resistance to Barley mild mosaic virus. Plant Pathology 53: 161-169

Molina-Cano JL, Moralejo M, Igartua E, Romagosa I (1999). Further evidence supporting Morocco as a center of origin of barley. Theo Appl Genet 98: 912-918

Molina-Cano JL, Russell JR, Moralejo MA, Escacena JL, Arias G, Powell W (2005) Chloroplast DNA microsatellite analysis supports a polyphyletic origin for barley. Theor Appl Genet 110: 613-619

Mueller KJ, Valè G, Enneking D (2003) Selection of resistant spring barley accessions after infection with leaf stripe (*Pyrenophora graminea*) under organic farming conditions in Germany and by sandwich test. J Plant Path 85: 9-14

Murphy PJ, Witcombe JR (1986) Covered and naked barleys from the Himalaya, 1. Evidence of multivariate differences between the two types. Theor Appl Genet 71: 730-735

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590

Nissan-Azzouz F, Graner A, Friedt W, Ordon F (2005) Fine-mapping of the BaMMV, BaYMV-1 and BaYMV-2 resistance of barley (*Hordeum vulgare*) accession 'PI1963'. Theor Appl Genet 110: 212-218

Nomura K, Kashiwazaki S, Hibino H, Inoue T, Nakata E, Tsuzaki Y, Okuyama S (1996) Biological and serological properties of strains of barley mild mosaic virus. J Phytopathol 144: 103-107

Ordon F, Ahlemeyer J, Werner K, Köhler W, Friedt W (2005) Molecular assessment of genetic diversity in winter barley and its use in breeding. Euphytica 146: 21-28

Ordon F, Bauer E, Friedt W, Graner A (1995) Marker-based selection for the *ym4* BaMMV-resistance gene in barley using RAPDs. Agronomie 15: 481-485

Ordon F, Friedt W (1993) Mode of inheritance and genetic diversity of BaMMV resistance of exotic barley germplasm carrying genes different from '*ym4*'. Theor Appl Genet 86: 229-233

Ordon F, Friedt W, Scheurer K, Pellio B, Werner K, Neuhaus G, Huth W, Habekuss A, Graner A (2004) Molecular markers in breeding for virus resistance in barley. J Appl Genet 45: 145-159

Ordon F, Götz R, Friedt W (1993) Genetic stocks resistant to barley yellow mosaic viruses (BaMMV, BaYMV, BaYMV-2) in Germany. Barley Genet Newslett 22: 46-49

Ordon F, Schimann A, Friedt W (1997) Assessment of the genetic relatedness of barley accessions (*Hordeum vulgare* s.l.) resistant to soil-borne mosaic-inducing viruses (BaMMV, BaYMV, BaYMV-2) using RAPDs. Theor Appl Genet 94: 325-330

Ortiz R, Nurminiemi M, Madsen S, Rognli OA, Bjørnstad Å (2002) Genetic gains in Nordic spring barley breeding over sixty years. Euphytica 126: 283-289

Patto MCV, Satovic Z, Pego S, Fevereiro P (2004) Assessing the genetic diversity of Portuguese maize germplasm using microsatellite markers. Euphytica 137: 63-72

Pellio B, Streng S, Bauer E, Stein N, Perovic D, Schiemann A, Friedt W, Ordon F, Graner A (2005) High-resolution mapping of the *Rym4/Rym5* locus conferring resistance to the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2) in barley (*Hordeum vulgare* ssp. *vulgare* L.). Theor Appl Genet 110: 283-293

Pickering R, Johnston PA (2005) Recent progress in barley improvement using wild species of *Hordeum*. Cytogenet Genome Res 109: 344-349

Poehlman JM (1979) Breeding field crops, 2nd edition, AVI Publishing, Westport

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945-959

Putkonen JK (2004) Continuous snow and rain data at 500 to 4400 m altitude near Annapurna, Nepal, 1999–2001. Arctic, Antarctic, and Alpine Research 36: 244-248

Ramsay L, Macaulay M, Ivanissevich SD, MacLean K, Cardle L, Fuller J, Edwards KJ, Tuvesson S, Morgante M, Massari A, Maestri E, Marmiroli N, Sjakste T, Ganal M, Powell W, Waugh R (2000). A simple sequence repeat-based linkage map of barley. Genetics 156: 1997-2005

Rao RV, Hodgkin T (2002) Genetic diversity and conservation and utilization of plant genetic resources. Plant cell, Tissue and Organ Culture 68: 1-19

Remington DL, Thornsberry JM, Matsuoka Y, Wilson LM, Whitt SR, Doebley J, Kresovich S, Goodman MM, Buckler ES (2001) Structure of linkage disequilibrium and phenotypic associations in the maize genome. PNAS 98: 11479-11484

Rice WR (1989) Analysing tables of statistical tests. Evolution 43: 223-225

Rimpau I, Smith DB, Flavell RB (1980) Sequence organization in barley and oats chromosomes revealed by interspecies DNA/DNA hybridization. Heredity 44: 131-149

Rohlf FJ (2000) NTSYS-pc numerical taxonomy and multivariate analysis system, version 2.1. Exeter Publications, New York

Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. Genetics 145: 1219-1228

Ruge B, Linz A, Habekuß A, Flath K, Wehling P (2004) Introgression and mapping of novel resistance genes from the secondary genepool of barley, *Hordeum bulbosum*. Proceedings of the 9th International Barley Genetics Symposium, 20-26 June 2004, Brno, Czech Republic, pp 729-736

Ruge B, Linz A, Pickering G, Greif P, Wehling P (2003) Mapping of *Rym14*^{HB}, a gene introgressed from *Hordeum bulbosum* and conferring resistance to BaMMV and BaYMV in barley. Theor Appl Genet 107: 965-971

Russell JR, Booth A, Fuller JD, Baum M, Ceccarelli S, Grando S, Powell W (2003) Patterns of polymorphism detected in the chloroplast and nuclear genomes of barley landraces sampled from Syria and Jordan. Theor Appl Genet 107: 413-421

Russell JR, Ellis RP, Thomas WTB, Waugh R, Provan J, Booth A, Fuller J, Lawrence P, Young G, Powell W (2000) A retrospective analysis of spring barley germplasm development from 'foundation genotypes' to currently successful cultivars. Mol Breed 6: 553-568

Russell JR, Fuller JD, Macaulay M, Hatz BG, Jahoor A, Powell W, Waugh R (1997) Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. Theor Appl Genet 95: 714-722

Saeki K, Miyazaki C, Hirota N, Saito A, Ito K, Konishi T (1999) RFLP mapping of BaYMV resistance gene *rym3* in barley (*Hordeum vulgare*). Theor Appl Genet 99: 727-732

Saghai Maroof MA, Allard RW, Zhang Q (1990) Genetic diversity and ecogeographical differentiation among ribosomal DNA alleles in wild and cultivated barley. Proc Natl Acad Sci USA 87: 8486-8490

Saghai Maroof MA, Biyashev RM, Yang GP, Zhangf Q, Allard RW (1994) Extraordinarily polymorphic microsatellite DNA in barley: Species diversity, chromosomal locations, and population dynamics. Proc Natl Acad Sci USA 91: 5466-5470

Scheurer KS, Friedt W, Huth W, Waugh R, Ordon F (2001) QTL analysis of tolerance to a German strain of BYDV-PAV in barley (*Hordeum vulgare* L.). Theor Appl Genet 103: 1074-1083

Schmierer DA, Kandemir N, Kudrna DA, Jones BL, Ullrich SE, Kleinhofs A (2004) Molecular marker-assisted selection for enhanced yield in malting barley. Molecular Breeding 14: 463-473

Schoen DJ, Brown AHD (1991) Intraspecific variation in population gene diversity and effective population size correlates with the mating system in plants. Proc Natl Acad Sci USA 88: 4494-4497

Shao Q, Chang-sen L, Chiren B (1982) Origin and evolution of cultivated barley: Wild barley from Western Szechuan and Tibet, China. Barley Genet Newslett 12: 37-42

Sharma KP, Dahal KR (1991) Exploitation of genetic yield potential of Uwa (*Hordeum vulgare* L.) Germplasm in the high altitude Himalayas. Annual Report, ISRP Activity No. 9.397, USAID

Sharma KP, Dahal KR, Basta BK (1994) Genetic diversity of Nepalese naked barley and possibility of yield improvement. Proceedings of the IInd National Conference on Science and Technology, 1994, Kathmandu. Royal Nepal Acad Sci Tech (RONAST) Kathmandu pp 231-237

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139: 457-462

Stein N, Perovic D, Kumlehn J, Pellio B, Stracke S, Streng S, Ordon F, Graner A (2005) The eukaryotic translation initiation factor 4E confers multiallelic recessive Bymovirus resistance in *Hordeum vulgare* (L.). The Plant Journal 42: 912-922

Stich B, Melchinger AE, Frisch M, Maurer HP, Heckenberger M, Reif JC (2005) Linkage disequilibrium in European elite maize germplasm investigated with SSRs. Theor Appl Genet 111: 723-730

Stoll C, Pellio B, Werner K, Uptmoor R, Waugh R, Friedt W, Ordon F (2002) Genetische Diversität von *Hordeum vulgare* s.l. ermittelt anhand von SSRs. Vortr Pflanzenzüchtg 54: 239-242

Strelchenko P, Kovalyova O, Okuno K (1999) Genetic differentiation and distribution of barley germplasm based on RAPD markers. Genet Resour Crop Evol 46: 193-205

Struss D, Plieske (1998) The use of microsatellite markers for detection of genetic diversity in barley populations. Theor Appl Genet 97: 308-315

Sun L, Wang X (1999) Genetic diversity of Chinese hull-less barley germplasm and its utilization. Plant Genet Resour Newsletter 120: 55-57

Takahashi R (1955) The origin and evolution of cultivated barley. Adv Genet 7: 227-266

Takahashi R, Hayashi J, Inouye T, Moriya I, Hirao C (1973) Studies on resistance to yellow mosaic disease in barley. I. Tests for varietal reactions and genetic analysis of resistance to the disease. Ber Ohara Inst 16: 1-17

Taketa S, Kikuchi S, Awayama T, Yamamoto S, Ichii M, Kawasaki S (2004) Monophyletic origin of naked barley inferred from molecular analyses of a marker closely linked to the naked caryopsis gene (*nud*). Theor Appl Genet 108: 1236-1242

Tanno K, Takeda K (2004) On the origin of six-rowed barley with brittle rachis, agriocrithon [*Hordeum vulgare* ssp. *vulgare* f. *agriocrithon* (Åberg) Bowd.], based on a DNA marker closely linked to the *vrs1* (six-row gene) locus. Theor Appl Genet 110: 145-150

Tanno K, Taketa S, Takeda K, Komatsuda T (2002) A DNA marker closely linked to the *vrs1* locus (row type gene) indicates multiple origins of six-rowed cultivated barley (*Hordeum vulgare* L.). Theor Appl Genet 104: 54-60

Terzi V, Pecchioni N, Faccioli P, Kucera L, Stanca AM (2001) Phyletic relationships within the genus *Hordeum* using PCR-based markers. Genet Resour Crop Evol 48: 447-458

Thomas WTB (2003) Prospects for molecular breeding of barley. Ann Appl Biol 142: 1-12

Toojinda T, Baird E, Booth A, Broers L, Hayes P, Powell W, Thomas W, Vivar H, Young G (1998) Introgression of quantitative trait loci (QTLs) determining stripe rust resistance in barley: an example of marker-assisted line development. Theor Appl Genet 96: 123-131

Toyama A, Kusaba T (1970) Transmission of soil-borne barley yellow mosaic virus. 2. *Polymyxa graminis* Led. as vector. Ann Phytopathol Soc Japan 36: 223-229

Treuren R van, Hintum T JL van (2001) Identification of intra-accession genetic diversity in selfing crops using AFLP markers: implications for collection management. Genet Resour Crop Evol 48: 287-295

Uptmoor R, Wenzel W, Friedt W, Donaldson G, Ayisi K, Ordon F (2003) Comparative analysis on the genetic relatedness of *Sorghum bicolour* accessions from Southern Africa by RAPDs, AFLPs and SSRs. Theor Appl Genet 106: 1316-1325

Valkoun J, Konopka J (2004) Global inventory of barley genetic resources. Proceedings of the 9th International Barley Genetics Symposium, 2004, Brno, Czech Republic pp 31-38

Weber JL (1990) Informativeness of human $(dC\text{-}dA)_n \cdot (dG\text{-}dT)_n$ polymorphisms. Genomics 7: 524-530

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38: 1358-1370

Wenzl P, Carling J, Kudrna D, Jaccoud D, Huttner E, Kleinhofs A, Kilian A (2004) Diversity Arrays Technology (DArT) for whole-genome profiling of barley. PNAS 101: 9915-9920

Werner K, Friedt W, Laubach E, Waugh R, Ordon F (2003a) Dissection of resistance to soil-borne yellow mosaic inducing viruses of barley (BaMMV, BaYMV, BaYMV-2) in a complex breeders cross by SSRs and simultaneous mapping of BaYMV/BaYMV-2 resistance of 'Chikurin Ibaraki 1'. Theor Appl Genet 106: 1425-1432

Werner K, Friedt W, Ordon F (2005) Strategies for pyramiding resistance genes against the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2). Molecular Breeding 16: 45-55

Werner K, Rönicke S, Le Gouis J, Friedt W, Ordon F (2003b) Mapping of a new BaMMV-resistance gene derived from the variety 'Taihoku A'. J Plant Dis Prot 110: 304-311

Witcombe JR, Murphy PJ (1986) Covered and naked barleys from the Himalaya, 2. Why do they differ from each other so extensively? Theor Appl Genet 71: 736-741

Wright S (1951) The genetical structure of populations. Ann Eugen 15: 323-354

Xu TW (1982) Origin and evolution of cultivated barley in China. Acta Genet Sin 9: 440-446

Yeh FC, Yang RC, Boyle T (1999) POPGENE version 1.32. Microsoft window-based freeware for population genetic analysis. University of Alberta, Edmonton, Alberta, Canada http:// www.ualberta.ca/~fyeh/pr01.htm

Yu SB, Xu WJ, Vijayakumar CHM, Ali J, Fu BY, Xu JL, Jiang YZ, Marghirang R, Domingo J, Aquino C, Virmani SS, Li ZK (2003) Molecular diversity and multilocus organization of the parental lines used in the International Rice Molecular Breeding Program. Theor Appl Genet 108: 131-140

Zhou H, Xie Z, Ge S (2003) Microsatellite analysis of genetic diversity and population genetic structure of a wild rice (*Oryza rufipogon* Griff.) in China. Theor Appl Genet 107: 332-339

Zohary D, Hopf M (1993) Domestication of plants in Old World. The origin and spread of cultivated plants in West Asia, Europe and the Nile Valley. Clarendon Press, Oxford, England

Zohary D, Hopf M (2000) Domestication of plants in the Old World: the origin and spread of cultivated plants in West Asia, Europe and the Nile Valley. Clarendon Press, Oxford

Appendix-I Genetic similarity coefficient (DICE)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1 Ann BC-1																												
2 Ann BC-2	0.77																											
3 Bimtakothi-1	0.68	0.66																										
4 Bimtakothi-2	0.68	0.61	0.80																									
5 Bimtakothi-3	0.54	0.58	0.74	0.58																								
6 Bimtakothi-4	0.70	0.64	0.73	0.84	0.54																							
7 Bimtakothi-5	0.66	0.59	0.80	0.82	0.58	0.75																						
8 Bimtakothi-9	0.55	0.57	0.77	0.61	0.94	0.59	0.61																					
9 Bimtakothi-10	0.54	0.58	0.74	0.70	0.56	0.76	0.79	0.61																				
10 Bimtakothi-11	0.55	0.59	0.73	0.59	0.94	0.55	0.61	0.93	0.56																			
11 Bimtakothi-12	0.64	0.61	0.70	0.77	0.61	0.77	0.73	0.64	0.70	0.61																		
12 Bimtakothi-13	0.59	0.52	0.61	0.66	0.49	0.68	0.59	0.52	0.61	0.50	0.66																	
13 Chame-2	0.59	0.61	0.66	0.73	0.54	0.68	0.73	0.55	0.67	0.50	0.70	0.61																
14 Chame-3	0.59	0.52	0.61	0.66	0.47	0.68	0.61	0.50	0.61	0.50	0.66	0.86	0.59															
15 Chame-8	0.45	0.43	0.56	0.54	0.53	0.52	0.49	0.56	0.56	0.52	0.49	0.61	0.54	0.56														
16 Chame-9	0.43	0.36	0.43	0.48	0.40	0.48	0.48	0.41	0.47	0.41	0.50	0.57	0.48	0.55	0.67													
17 Chame-11	0.57	0.48	0.57	0.59	0.43	0.57	0.64	0.43	0.58	0.45	0.57	0.61	0.64	0.64	0.45	0.48												
18 Chame-12	0.64	0.52	0.66	0.68	0.49	0.70	0.64	0.52	0.63	0.48	0.57	0.61	0.66	0.64	0.49	0.48	0.66											
19 Chame-13	0.66	0.55	0.61	0.73	0.49	0.70	0.68	0.50	0.58	0.48	0.66	0.66	0.75	0.66	0.54	0.45	0.70	0.66										
20 Chame-14	0.55	0.50	0.55	0.64	0.49	0.59	0.55	0.50	0.54	0.52	0.59	0.57	0.61	0.57	0.58	0.61	0.61	0.50	0.61									
21 Dhumpu-2	0.45	0.39	0.43	0.45	0.43	0.45	0.48	0.43	0.45	0.45	0.50	0.50	0.45	0.55	0.61	0.84	0.48	0.45	0.45	0.64								
22 Ghara-1	0.34	0.32	0.30	0.32	0.47	0.30	0.34	0.48	0.31	0.50	0.39	0.32	0.32	0.34	0.40	0.48	0.36	0.34	0.32	0.39	0.52							
23 Ghara-2	0.39	0.32	0.41	0.41	0.56	0.41	0.39	0.57	0.36	0.59	0.43	0.39	0.34	0.41	0.45	0.43	0.36	0.41	0.39	0.48	0.52	0.77						
24 Gho-1	0.50	0.48	0.55	0.57	0.56	0.55	0.48	0.57	0.49	0.57	0.59	0.57	0.55	0.55	0.63	0.59	0.45	0.52	0.52	0.55	0.55	0.36	0.50					
25 Gho-2	0.57	0.50	0.64	0.59	0.61	0.59	0.59	0.61	0.56	0.61	0.59	0.57	0.57	0.52	0.58	0.64	0.52	0.61	0.57	0.59	0.55	0.34	0.48	0.80				
26 Gho-3	0.50	0.45	0.55	0.64	0.45	0.59	0.55	0.48	0.45	0.48	0.59	0.57	0.52	0.57	0.56	0.55	0.50	0.55	0.52	0.48	0.50	0.39	0.45	0.64	0.61			
27 Jomson-1	0.48	0.39	0.48	0.52	0.40	0.52	0.50	0.41	0.49	0.41	0.55	0.61	0.50	0.57	0.63	0.80	0.55	0.52	0.48	0.68	0.68	0.43	0.43	0.64	0.61	0.59		
28 Jomson-2	0.50	0.41	0.48	0.52	0.45	0.50	0.52	0.45	0.47	0.48	0.57	0.59	0.52	0.59	0.67	0.77	0.55	0.50	0.52	0.73	0.75	0.45	0.52	0.66	0.64	0.61	0.86	
29 Kagbeni-3	0.48	0.39	0.45	0.50	0.43	0.50	0.50	0.43	0.47	0.45	0.55	0.57	0.50	0.61	0.63	0.77	0.52	0.50	0.50	0.73	0.77	0.45	0.55	0.64	0.61	0.59	0.84	0.93
30 Kagbeni-5	0.48	0.39	0.45	0.55	0.43	0.48	0.50	0.43	0.45	0.45	0.55	0.57	0.50	0.64	0.65	0.80	0.52	0.48	0.52	0.70	0.80	0.43	0.50	0.64	0.61	0.61	0.80	0.86
31 Lih Dhanra Gal	0.61	0.55	0.52	0.52	0.47	0.52	0.52	0.48	0.52	0.50	0.57	0.52	0.57	0.57	0.47	0.48	0.52	0.55	0.55	0.57	0.61	0.41	0.45	0.45	0.45	0.45	0.52	0.57
32 N6	0.39	0.43	0.46	0.37	0.49	0.39	0.46	0.50	0.43	0.52	0.43	0.35	0.39	0.39	0.43	0.46	0.39	0.37	0.37	0.37	0.54	0.50	0.54	0.43	0.43	0.52	0.43	0.48
33 N12	0.39	0.41	0.45	0.41	0.49	0.41	0.45	0.48	0.43	0.52	0.45	0.36	0.41	0.39	0.49	0.48	0.43	0.39	0.41	0.45	0.55	0.48	0.57	0.50	0.48	0.55	0.45	0.52
34 Naked-304	0.45	0.43	0.52	0.43	0.52	0.45	0.50	0.52	0.52	0.55	0.48	0.43	0.50	0.45	0.52	0.59	0.52	0.48	0.43	0.61	0.64	0.36	0.43	0.52	0.61	0.41	0.57	0.66
35 Nepal 1	0.43	0.31	0.40	0.43	0.47	0.40	0.43	0.47	0.38	0.49	0.40	0.36	0.36	0.43	0.42	0.52	0.40	0.43	0.45	0.52	0.63	0.56	0.72	0.47	0.54	0.43	0.47	0.56
36 Nepal 2	0.43	0.32	0.43	0.45	0.49	0.43	0.43	0.50	0.40	0.50	0.43	0.39	0.39	0.36	0.45	0.55	0.43	0.45	0.45	0.52	0.57	0.57	0.70	0.52	0.61	0.48	0.52	0.57
37 Nepal 3	0.39	0.36	0.45	0.39	0.54	0.43	0.43	0.55	0.47	0.55	0.41	0.45	0.41	0.41	0.63	0.57	0.41	0.45	0.41	0.45	0.52	0.52	0.55	0.59	0.59	0.52	0.55	0.57

Appendix-I Cont.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
38 Nepal 4	0.36	0.36	0.45	0.39	0.54	0.43	0.41	0.55	0.47	0.55	0.41	0.45	0.41	0.45	0.63	0.55	0.39	0.43	0.39	0.45	0.57	0.52	0.57	0.57	0.52	0.45	0.50	0.52
39 Nepal 5	0.36	0.36	0.45	0.40	0.53	0.43	0.40	0.54	0.47	0.54	0.43	0.43	0.40	0.45	0.64	0.56	0.38	0.43	0.40	0.47	0.58	0.49	0.54	0.58	0.54	0.47	0.49	0.52
40 Nepal 6	0.41	0.41	0.48	0.43	0.52	0.48	0.43	0.52	0.52	0.52	0.43	0.48	0.43	0.50	0.61	0.57	0.41	0.48	0.43	0.50	0.59	0.45	0.52	0.57	0.57	0.45	0.52	0.55
41 Nepal 7	0.33	0.30	0.43	0.39	0.54	0.37	0.39	0.54	0.43	0.54	0.37	0.37	0.37	0.39	0.54	0.52	0.39	0.39	0.37	0.46	0.54	0.54	0.65	0.59	0.59	0.46	0.48	0.52
42 Ngyak 1	0.52	0.45	0.57	0.64	0.54	0.59	0.57	0.57	0.47	0.57	0.57	0.52	0.50	0.52	0.47	0.50	0.52	0.55	0.50	0.50	0.50	0.36	0.43	0.68	0.70	0.73	0.57	0.55
43 Ngyak 2	0.43	0.41	0.50	0.50	0.45	0.52	0.50	0.48	0.49	0.48	0.43	0.52	0.43	0.52	0.63	0.50	0.50	0.52	0.48	0.43	0.45	0.34	0.43	0.66	0.61	0.68	0.57	0.50
44 Ngyak 3	0.48	0.43	0.55	0.55	0.58	0.52	0.50	0.59	0.52	0.59	0.64	0.50	0.50	0.50	0.52	0.52	0.50	0.50	0.50	0.55	0.50	0.36	0.45	0.80	0.77	0.57	0.57	0.59
45 Ngyak 4	0.52	0.45	0.59	0.55	0.63	0.55	0.55	0.64	0.54	0.64	0.59	0.48	0.52	0.45	0.56	0.55	0.50	0.59	0.52	0.57	0.52	0.39	0.52	0.82	0.89	0.59	0.55	0.59
46 Tilman camp 7	0.57	0.50	0.50	0.57	0.45	0.59	0.55	0.43	0.61	0.43	0.61	0.70	0.59	0.75	0.52	0.55	0.68	0.64	0.64	0.57	0.52	0.30	0.41	0.55	0.50	0.48	0.59	0.64
47 Ngyak 10	0.43	0.43	0.48	0.52	0.52	0.50	0.50	0.52	0.49	0.52	0.61	0.52	0.55	0.50	0.54	0.52	0.50	0.45	0.50	0.55	0.50	0.50	0.55	0.70	0.55	0.55	0.57	0.64
48 Ngyak 11	0.65	0.61	0.54	0.58	0.47	0.56	0.56	0.47	0.51	0.47	0.58	0.56	0.56	0.58	0.51	0.52	0.45	0.54	0.58	0.52	0.56	0.34	0.40	0.54	0.52	0.52	0.52	0.54
49 Ngyak 12	0.55	0.50	0.66	0.61	0.54	0.55	0.59	0.57	0.52	0.55	0.52	0.52	0.48	0.50	0.56	0.43	0.41	0.45	0.52	0.43	0.43	0.32	0.43	0.57	0.55	0.77	0.50	0.50
50 Phalatey	0.50	0.39	0.50	0.57	0.47	0.52	0.55	0.48	0.49	0.48	0.57	0.59	0.52	0.57	0.61	0.70	0.55	0.45	0.55	0.73	0.70	0.36	0.39	0.61	0.66	0.55	0.73	0.82
51 Philem 1	0.43	0.48	0.45	0.48	0.45	0.48	0.43	0.48	0.47	0.45	0.52	0.48	0.45	0.45	0.54	0.43	0.34	0.39	0.43	0.41	0.45	0.43	0.50	0.61	0.48	0.55	0.43	0.43
52 Philem 2	0.46	0.51	0.48	0.51	0.45	0.51	0.48	0.46	0.48	0.44	0.53	0.46	0.48	0.48	0.50	0.44	0.39	0.46	0.39	0.37	0.48	0.41	0.44	0.57	0.44	0.53	0.44	0.41
53 Philem 3	0.46	0.51	0.48	0.53	0.43	0.48	0.48	0.44	0.45	0.44	0.51	0.46	0.46	0.48	0.52	0.46	0.39	0.46	0.41	0.39	0.51	0.41	0.44	0.60	0.46	0.55	0.44	0.41
54 Pisang 4	0.64	0.68	0.70	0.66	0.56	0.68	0.64	0.57	0.61	0.52	0.66	0.66	0.75	0.64	0.52	0.45	0.50	0.68	0.64	0.48	0.43	0.32	0.34	0.50	0.52	0.50	0.45	0.45
55 Pisang 5	0.47	0.47	0.56	0.61	0.47	0.56	0.56	0.49	0.58	0.45	0.58	0.52	0.54	0.49	0.60	0.56	0.52	0.54	0.56	0.52	0.52	0.34	0.34	0.54	0.61	0.54	0.54	0.49
56 Pisang 6	0.40	0.45	0.45	0.52	0.40	0.47	0.52	0.40	0.47	0.43	0.56	0.43	0.47	0.43	0.44	0.47	0.49	0.43	0.49	0.52	0.45	0.34	0.31	0.47	0.56	0.52	0.49	0.49
57 Pisang 7	0.49	0.49	0.54	0.52	0.49	0.49	0.56	0.49	0.53	0.49	0.56	0.45	0.47	0.43	0.47	0.40	0.45	0.43	0.54	0.52	0.45	0.25	0.31	0.52	0.61	0.43	0.43	0.47
58 Pisang 8	0.54	0.52	0.56	0.56	0.53	0.56	0.58	0.56	0.58	0.54	0.63	0.54	0.52	0.49	0.53	0.54	0.49	0.47	0.61	0.58	0.56	0.34	0.40	0.56	0.67	0.47	0.47	0.52
59 Pisang 9	0.50	0.52	0.57	0.57	0.56	0.55	0.59	0.59	0.56	0.55	0.66	0.50	0.61	0.48	0.49	0.52	0.50	0.55	0.55	0.55	0.52	0.36	0.39	0.59	0.68	0.52	0.55	0.59
60 Pork 1	0.43	0.36	0.43	0.50	0.40	0.50	0.48	0.43	0.40	0.41	0.48	0.50	0.45	0.52	0.56	0.52	0.48	0.50	0.50	0.45	0.48	0.34	0.43	0.57	0.52	0.66	0.55	0.52
61 Pork 2	0.43	0.36	0.43	0.49	0.40	0.49	0.47	0.40	0.40	0.40	0.45	0.52	0.45	0.52	0.56	0.52	0.52	0.47	0.45	0.47	0.47	0.38	0.43	0.54	0.47	0.67	0.58	0.56
62 Sama 1	0.31	0.38	0.40	0.31	0.42	0.36	0.36	0.43	0.40	0.45	0.40	0.43	0.36	0.43	0.44	0.38	0.36	0.36	0.31	0.38	0.45	0.45	0.52	0.45	0.38	0.43	0.43	0.45
63 Sama 2	0.34	0.38	0.36	0.29	0.33	0.34	0.34	0.36	0.36	0.34	0.40	0.38	0.34	0.38	0.36	0.40	0.34	0.34	0.27	0.34	0.40	0.45	0.40	0.31	0.27	0.31	0.40	0.38
64 Sama 3	0.55	0.52	0.50	0.50	0.43	0.57	0.48	0.45	0.47	0.43	0.52	0.48	0.50	0.50	0.43	0.39	0.41	0.48	0.43	0.43	0.43	0.27	0.39	0.39	0.39	0.43	0.39	0.39
65 Sama 4	0.43	0.43	0.48	0.36	0.49	0.43	0.48	0.52	0.47	0.50	0.50	0.41	0.41	0.43	0.52	0.43	0.32	0.41	0.36	0.34	0.50	0.57	0.50	0.45	0.41	0.43	0.43	0.45
66 Sama 6	0.36	0.40	0.34	0.31	0.31	0.34	0.36	0.31	0.33	0.31	0.36	0.38	0.34	0.43	0.36	0.38	0.34	0.36	0.27	0.34	0.43	0.43	0.43	0.31	0.27	0.31	0.40	0.38
67 Sama 8	0.61	0.55	0.52	0.52	0.47	0.52	0.52	0.50	0.47	0.48	0.59	0.48	0.57	0.50	0.47	0.48	0.45	0.50	0.52	0.50	0.55	0.34	0.39	0.45	0.48	0.43	0.48	0.50
68 Sama 9	0.55	0.50	0.50	0.50	0.43	0.52	0.52	0.43	0.49	0.43	0.55	0.50	0.50	0.55	0.49	0.50	0.45	0.48	0.57	0.43	0.52	0.30	0.36	0.50	0.48	0.45	0.50	0.52
69 Sikha 1	0.36	0.32	0.39	0.36	0.49	0.39	0.45	0.52	0.38	0.50	0.39	0.34	0.41	0.36	0.40	0.43	0.34	0.36	0.36	0.41	0.52	0.61	0.70	0.41	0.39	0.39	0.39	0.43
70 Sikha 2	0.39	0.32	0.39	0.36	0.49	0.39	0.45	0.52	0.38	0.50	0.39	0.34	0.41	0.39	0.40	0.43	0.34	0.36	0.36	0.41	0.52	0.61	0.70	0.41	0.39	0.39	0.39	0.43
71 Sikha 4	0.43	0.34	0.45	0.47	0.49	0.45	0.43	0.52	0.40	0.49	0.43	0.40	0.40	0.45	0.47	0.52	0.36	0.43	0.43	0.52	0.58	0.56	0.72	0.49	0.54	0.45	0.47	0.52
72 Sikha 5	0.36	0.36	0.43	0.41	0.56	0.41	0.45	0.57	0.40	0.52	0.41	0.36	0.45	0.34	0.45	0.45	0.39	0.39	0.39	0.43	0.48	0.64	0.68	0.43	0.43	0.43	0.43	0.43
73 Sikha 6	0.36	0.34	0.41	0.41	0.52	0.41	0.43	0.55	0.40	0.52	0.41	0.36	0.43	0.41	0.45	0.45	0.34	0.36	0.36	0.45	0.52	0.61	0.73	0.45	0.41	0.41	0.39	0.41
74 Sikha 7	0.36	0.34	0.41	0.41	0.47	0.41	0.43	0.50	0.40	0.48	0.41	0.36	0.43	0.39	0.43	0.45	0.34	0.34	0.36	0.45	0.50	0.61	0.70	0.41	0.41	0.36	0.39	0.41
75 Sikha 8	0.43	0.34	0.45	0.43	0.51	0.40	0.40	0.52	0.40	0.52	0.43	0.36	0.36	0.40	0.47	0.54	0.38	0.43	0.43	0.52	0.58	0.58	0.72	0.54	0.61	0.43	0.52	0.54

Appendix-I Cont.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
76 Sipche 2	0.43	0.43	0.57	0.50	0.63	0.48	0.48	0.66	0.52	0.61	0.45	0.52	0.50	0.52	0.70	0.52	0.41	0.45	0.48	0.45	0.55	0.52	0.59	0.59	0.55	0.50	0.50	0.50
77 Sipche 3	0.48	0.43	0.52	0.50	0.61	0.50	0.45	0.64	0.47	0.61	0.50	0.50	0.48	0.50	0.65	0.52	0.39	0.45	0.43	0.45	0.55	0.57	0.61	0.66	0.57	0.55	0.52	0.57
78 Sipche 4	0.45	0.43	0.55	0.52	0.63	0.50	0.45	0.66	0.47	0.61	0.50	0.50	0.50	0.50	0.67	0.52	0.39	0.48	0.45	0.45	0.55	0.57	0.61	0.66	0.57	0.55	0.52	0.57
79 Sipche 6	0.45	0.43	0.55	0.52	0.63	0.50	0.45	0.66	0.47	0.61	0.50	0.50	0.50	0.50	0.67	0.52	0.39	0.48	0.45	0.45	0.55	0.57	0.61	0.66	0.57	0.55	0.52	0.57
80 Sipche 7	0.45	0.43	0.55	0.52	0.63	0.50	0.45	0.66	0.47	0.61	0.50	0.50	0.50	0.50	0.67	0.52	0.39	0.48	0.45	0.45	0.55	0.57	0.61	0.66	0.57	0.55	0.52	0.57
81 Sipche 9	0.45	0.43	0.55	0.52	0.63	0.50	0.45	0.66	0.47	0.61	0.50	0.50	0.50	0.50	0.67	0.52	0.39	0.48	0.45	0.45	0.55	0.57	0.61	0.66	0.57	0.55	0.52	0.57
82 Sipche 11	0.45	0.43	0.54	0.52	0.62	0.49	0.45	0.65	0.47	0.61	0.49	0.49	0.49	0.49	0.67	0.52	0.38	0.47	0.45	0.45	0.54	0.56	0.61	0.65	0.56	0.54	0.52	0.56
83 Sipche 12	0.38	0.47	0.47	0.38	0.47	0.38	0.40	0.47	0.44	0.45	0.43	0.45	0.45	0.43	0.53	0.45	0.38	0.38	0.36	0.43	0.47	0.45	0.49	0.49	0.43	0.38	0.47	0.47
84 Thangja 1	0.61	0.59	0.57	0.61	0.49	0.64	0.64	0.52	0.61	0.50	0.59	0.52	0.57	0.55	0.45	0.45	0.50	0.57	0.52	0.45	0.45	0.41	0.43	0.41	0.50	0.39	0.43	0.41
85 Thangja 2	0.64	0.48	0.64	0.64	0.49	0.64	0.64	0.50	0.58	0.48	0.57	0.61	0.61	0.61	0.54	0.52	0.66	0.82	0.68	0.50	0.50	0.34	0.36	0.52	0.59	0.50	0.52	0.52
86 Thangja 3	0.58	0.49	0.63	0.63	0.49	0.63	0.63	0.49	0.56	0.47	0.54	0.56	0.52	0.56	0.53	0.54	0.61	0.54	0.58	0.47	0.52	0.36	0.43	0.52	0.52	0.52	0.56	0.54
87 Thomje 2	0.34	0.40	0.38	0.36	0.33	0.40	0.40	0.34	0.40	0.34	0.40	0.40	0.38	0.38	0.38	0.38	0.34	0.34	0.31	0.38	0.40	0.40	0.40	0.36	0.29	0.34	0.40	0.38
88 Thomje 3	0.36	0.40	0.38	0.31	0.40	0.38	0.40	0.43	0.47	0.40	0.38	0.38	0.38	0.40	0.44	0.40	0.38	0.36	0.31	0.36	0.45	0.45	0.47	0.40	0.34	0.34	0.40	0.38
89 Thomje 4	0.34	0.41	0.41	0.32	0.45	0.36	0.39	0.48	0.45	0.45	0.43	0.41	0.41	0.43	0.49	0.45	0.34	0.36	0.32	0.41	0.50	0.48	0.55	0.48	0.41	0.32	0.45	0.43
90 Thomje 6	0.40	0.38	0.49	0.43	0.53	0.45	0.47	0.56	0.51	0.54	0.52	0.45	0.43	0.47	0.49	0.49	0.47	0.47	0.40	0.52	0.49	0.38	0.43	0.45	0.54	0.47	0.52	0.49
91 Thomje 7	0.34	0.41	0.41	0.32	0.45	0.34	0.39	0.45	0.43	0.45	0.41	0.41	0.41	0.43	0.52	0.45	0.34	0.39	0.32	0.39	0.50	0.48	0.57	0.50	0.43	0.34	0.43	0.48
92 Thonje 3	0.73	0.70	0.61	0.59	0.54	0.64	0.59	0.57	0.56	0.55	0.59	0.55	0.57	0.55	0.47	0.45	0.48	0.57	0.57	0.50	0.48	0.34	0.43	0.45	0.50	0.41	0.43	0.43
93 Thonje 4	0.59	0.59	0.59	0.59	0.54	0.59	0.64	0.55	0.65	0.52	0.64	0.59	0.59	0.59	0.65	0.57	0.52	0.52	0.57	0.52	0.55	0.39	0.41	0.52	0.57	0.48	0.52	0.55
94 Thonje 5	0.57	0.57	0.73	0.66	0.61	0.70	0.70	0.64	0.70	0.61	0.75	0.59	0.57	0.59	0.47	0.45	0.50	0.50	0.57	0.48	0.48	0.36	0.41	0.48	0.52	0.45	0.45	0.43
95 Thonje 6	0.67	0.55	0.74	0.71	0.64	0.64	0.71	0.64	0.64	0.62	0.67	0.62	0.57	0.62	0.50	0.48	0.57	0.57	0.64	0.55	0.53	0.34	0.39	0.51	0.57	0.53	0.55	0.57
96 Thonje 16	0.59	0.48	0.59	0.66	0.49	0.64	0.61	0.50	0.56	0.48	0.66	0.84	0.59	0.84	0.58	0.57	0.61	0.59	0.73	0.55	0.55	0.36	0.36	0.55	0.55	0.55	0.59	0.61
97 Thonje 18	0.59	0.45	0.57	0.57	0.54	0.55	0.59	0.52	0.52	0.52	0.61	0.57	0.64	0.57	0.52	0.50	0.59	0.61	0.59	0.68	0.55	0.36	0.43	0.59	0.66	0.45	0.57	0.61
98 Thonje 19	0.57	0.61	0.57	0.50	0.54	0.52	0.52	0.52	0.52	0.52	0.50	0.45	0.57	0.43	0.47	0.45	0.43	0.48	0.52	0.61	0.50	0.30	0.34	0.52	0.59	0.39	0.48	0.50
99 Thonje 21	0.40	0.31	0.43	0.45	0.40	0.43	0.45	0.40	0.44	0.40	0.43	0.52	0.45	0.49	0.62	0.76	0.45	0.45	0.45	0.58	0.70	0.45	0.45	0.58	0.61	0.49	0.67	0.67
100 Tilman camp 1	0.75	0.75	0.61	0.59	0.54	0.59	0.66	0.55	0.56	0.52	0.59	0.52	0.61	0.48	0.47	0.45	0.50	0.55	0.64	0.48	0.48	0.36	0.36	0.43	0.50	0.41	0.43	0.45
101 Tilman camp 8	0.66	0.50	0.61	0.61	0.49	0.61	0.61	0.50	0.56	0.48	0.55	0.59	0.59	0.59	0.52	0.48	0.68	0.80	0.66	0.50	0.48	0.34	0.36	0.50	0.57	0.45	0.50	0.50
102 Tsumje 1	0.47	0.38	0.43	0.49	0.51	0.45	0.47	0.52	0.44	0.52	0.49	0.49	0.45	0.54	0.53	0.56	0.43	0.45	0.49	0.56	0.61	0.49	0.54	0.52	0.43	0.45	0.56	0.61
103 Tsumje 2	0.47	0.36	0.49	0.56	0.49	0.54	0.54	0.49	0.47	0.49	0.56	0.54	0.52	0.52	0.51	0.52	0.56	0.49	0.56	0.52	0.49	0.38	0.47	0.61	0.54	0.61	0.56	0.58
104 Tukucha	0.45	0.34	0.43	0.49	0.40	0.45	0.47	0.40	0.42	0.40	0.52	0.54	0.47	0.56	0.60	0.72	0.47	0.47	0.49	0.67	0.74	0.40	0.49	0.67	0.63	0.56	0.74	0.85
105 Ulleri 9	0.45	0.31	0.45	0.49	0.49	0.45	0.47	0.52	0.44	0.47	0.49	0.45	0.45	0.47	0.56	0.61	0.43	0.49	0.49	0.58	0.65	0.56	0.65	0.47	0.52	0.43	0.58	0.65
106 Ulleri 21	0.45	0.32	0.45	0.48	0.49	0.43	0.45	0.52	0.43	0.48	0.48	0.43	0.43	0.45	0.54	0.59	0.41	0.48	0.48	0.57	0.68	0.59	0.68	0.45	0.50	0.41	0.57	0.64
107 Solu Uwa	0.36	0.34	0.43	0.45	0.52	0.39	0.50	0.52	0.38	0.50	0.43	0.32	0.48	0.36	0.40	0.45	0.41	0.43	0.41	0.43	0.50	0.59	0.64	0.39	0.41	0.43	0.43	0.45

Appendix-I Cont.

	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
30 Kagbeni-5	0.91																											
31 Lih Dhanra Gal	0.57	0.59																										
32 N6	0.50	0.50	0.48																									
33 N12	0.52	0.52	0.50	0.74																								
34 Naked-304	0.66	0.64	0.57	0.48	0.55																							
35 Nepal 1	0.58	0.65	0.49	0.56	0.56	0.54																						
36 Nepal 2	0.55	0.57	0.43	0.50	0.55	0.52	0.92																					
37 Nepal 3	0.55	0.55	0.45	0.52	0.48	0.57	0.56	0.64																				
38 Nepal 4	0.55	0.57	0.50	0.54	0.45	0.55	0.58	0.57	0.93																			
39 Nepal 5	0.54	0.58	0.49	0.54	0.47	0.54	0.60	0.58	0.90	0.97																		
40 Nepal 6	0.57	0.61	0.55	0.52	0.45	0.57	0.63	0.61	0.86	0.93	0.94																	
41 Nepal 7	0.54	0.59	0.39	0.58	0.52	0.52	0.80	0.78	0.74	0.80	0.82	0.80																
42 Ngyak 1	0.52	0.57	0.43	0.43	0.45	0.50	0.54	0.57	0.50	0.45	0.47	0.45	0.54															
43 Ngyak 2	0.48	0.50	0.39	0.46	0.50	0.48	0.47	0.50	0.61	0.57	0.58	0.57	0.57	0.70														
44 Ngyak 3	0.57	0.59	0.45	0.46	0.45	0.61	0.52	0.55	0.59	0.55	0.56	0.52	0.57	0.73	0.61													
45 Ngyak 4	0.57	0.55	0.43	0.46	0.50	0.64	0.56	0.64	0.61	0.55	0.56	0.55	0.61	0.73	0.64	0.89												
46 Tilman camp 7	0.64	0.59	0.52	0.37	0.39	0.48	0.40	0.41	0.41	0.43	0.43	0.48	0.41	0.45	0.45	0.48	0.48											
47 Ngyak 10	0.59	0.57	0.41	0.54	0.55	0.45	0.43	0.48	0.55	0.52	0.54	0.50	0.54	0.50	0.50	0.64	0.59	0.55										
48 Ngyak 11	0.56	0.61	0.79	0.47	0.43	0.47	0.47	0.43	0.49	0.52	0.51	0.56	0.41	0.47	0.40	0.45	0.47	0.58	0.43									
49 Ngyak 12	0.48	0.50	0.45	0.52	0.55	0.39	0.45	0.45	0.48	0.45	0.47	0.45	0.48	0.61	0.68	0.50	0.55	0.43	0.48	0.56								
50 Phalatey	0.77	0.80	0.50	0.41	0.43	0.61	0.52	0.52	0.50	0.50	0.52	0.55	0.52	0.57	0.45	0.61	0.61	0.64	0.57	0.54	0.50							
51 Philem 1	0.39	0.43	0.39	0.48	0.52	0.32	0.40	0.43	0.48	0.48	0.49	0.45	0.46	0.50	0.57	0.48	0.48	0.39	0.61	0.45	0.55	0.39						
52 Philem 2	0.44	0.46	0.39	0.48	0.44	0.34	0.39	0.37	0.46	0.51	0.50	0.46	0.46	0.53	0.60	0.46	0.44	0.46	0.53	0.45	0.51	0.37	0.80					
53 Philem 3	0.44	0.48	0.39	0.48	0.46	0.34	0.41	0.39	0.46	0.51	0.52	0.48	0.48	0.55	0.62	0.46	0.46	0.44	0.53	0.48	0.53	0.39	0.80	0.95				
54 Pisang 4	0.43	0.43	0.57	0.39	0.36	0.43	0.34	0.36	0.41	0.41	0.40	0.45	0.35	0.48	0.45	0.41	0.45	0.59	0.45	0.63	0.50	0.45	0.45	0.53	0.51			
55 Pisang 5	0.47	0.52	0.52	0.41	0.43	0.49	0.38	0.43	0.47	0.43	0.44	0.45	0.37	0.56	0.52	0.58	0.58	0.43	0.49	0.53	0.47	0.52	0.49	0.48	0.52	0.56		
56 Pisang 6	0.47	0.52	0.47	0.43	0.43	0.47	0.38	0.40	0.40	0.36	0.38	0.40	0.34	0.54	0.45	0.56	0.54	0.36	0.47	0.49	0.38	0.54	0.40	0.39	0.43	0.47	0.78	
57 Pisang 7	0.43	0.45	0.47	0.45	0.47	0.45	0.40	0.40	0.40	0.38	0.40	0.43	0.37	0.49	0.43	0.56	0.58	0.40	0.49	0.51	0.47	0.56	0.45	0.39	0.41	0.45	0.62	0.69
58 Pisang 8	0.49	0.52	0.54	0.43	0.47	0.49	0.47	0.47	0.47	0.45	0.47	0.47	0.41	0.52	0.45	0.61	0.63	0.45	0.49	0.56	0.49	0.56	0.52	0.43	0.43	0.49	0.76	0.60
59 Pisang 9	0.52	0.55	0.57	0.46	0.41	0.52	0.43	0.48	0.50	0.45	0.45	0.48	0.41	0.59	0.43	0.68	0.66	0.45	0.57	0.56	0.41	0.59	0.50	0.44	0.41	0.57	0.67	0.70
60 Pork 1	0.55	0.57	0.43	0.43	0.45	0.39	0.45	0.45	0.52	0.52	0.54	0.52	0.52	0.61	0.75	0.52	0.52	0.50	0.52	0.49	0.66	0.52	0.52	0.57	0.57	0.41	0.49	0.47
61 Pork 2	0.54	0.56	0.43	0.45	0.45	0.45	0.42	0.45	0.52	0.47	0.47	0.45	0.47	0.63	0.76	0.49	0.45	0.49	0.54	0.44	0.58	0.49	0.49	0.55	0.52	0.45	0.51	0.47
62 Sama 1	0.43	0.43	0.34	0.45	0.47	0.45	0.42	0.45	0.54	0.52	0.51	0.49	0.49	0.36	0.47	0.38	0.43	0.38	0.54	0.27	0.40	0.36	0.56	0.48	0.48	0.40	0.31	0.29
63 Sama 2	0.38	0.40	0.29	0.37	0.34	0.34	0.36	0.36	0.40	0.43	0.42	0.43	0.43	0.27	0.34	0.25	0.27	0.38	0.45	0.31	0.27	0.34	0.54	0.50	0.48	0.43	0.27	0.27
64 Sama 3	0.43	0.41	0.64	0.33	0.34	0.41	0.38	0.36	0.36	0.41	0.40	0.41	0.37	0.41	0.41	0.34	0.39	0.48	0.32	0.61	0.43	0.34	0.45	0.53	0.51	0.57	0.36	0.31
65 Sama 4	0.50	0.45	0.43	0.57	0.50	0.41	0.40	0.36	0.50	0.52	0.52	0.50	0.48	0.36	0.43	0.39	0.43	0.36	0.52	0.45	0.45	0.36	0.61	0.60	0.57	0.43	0.34	0.31

Appendix-I Cont.

	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
66 Sama 6	0.40	0.45	0.31	0.39	0.34	0.34	0.40	0.36	0.40	0.45	0.44	0.45	0.45	0.29	0.36	0.27	0.27	0.40	0.45	0.33	0.27	0.34	0.52	0.52	0.52	0.43	0.27	0.27
67 Sama 8	0.52	0.55	0.82	0.43	0.45	0.52	0.45	0.41	0.43	0.45	0.45	0.45	0.35	0.41	0.34	0.43	0.43	0.48	0.39	0.76	0.45	0.45	0.48	0.51	0.48	0.59	0.54	0.45
68 Sama 9	0.55	0.57	0.70	0.46	0.41	0.52	0.47	0.43	0.55	0.57	0.56	0.57	0.46	0.48	0.45	0.52	0.48	0.57	0.43	0.81	0.50	0.50	0.43	0.44	0.44	0.55	0.54	0.49
69 Sikha 1	0.45	0.45	0.48	0.59	0.57	0.45	0.70	0.66	0.50	0.52	0.52	0.50	0.63	0.41	0.41	0.39	0.41	0.34	0.45	0.43	0.43	0.39	0.41	0.44	0.44	0.39	0.34	0.29
70 Sikha 2	0.45	0.48	0.48	0.57	0.57	0.45	0.72	0.66	0.50	0.52	0.52	0.50	0.63	0.41	0.41	0.41	0.41	0.34	0.45	0.40	0.43	0.39	0.43	0.46	0.46	0.39	0.34	0.29
71 Sikha 4	0.54	0.58	0.45	0.52	0.52	0.45	0.82	0.79	0.47	0.52	0.53	0.56	0.69	0.47	0.43	0.47	0.52	0.40	0.45	0.44	0.45	0.49	0.45	0.43	0.45	0.38	0.40	0.38
72 Sikha 5	0.41	0.41	0.43	0.52	0.55	0.43	0.65	0.70	0.55	0.50	0.49	0.48	0.61	0.45	0.45	0.43	0.45	0.32	0.48	0.36	0.43	0.39	0.43	0.41	0.41	0.43	0.40	0.34
73 Sikha 6	0.43	0.48	0.45	0.54	0.57	0.43	0.72	0.68	0.50	0.55	0.56	0.55	0.67	0.43	0.43	0.41	0.43	0.36	0.48	0.40	0.43	0.39	0.48	0.48	0.51	0.41	0.36	0.31
74 Sikha 7	0.41	0.45	0.43	0.48	0.57	0.43	0.70	0.68	0.45	0.48	0.49	0.52	0.61	0.39	0.39	0.36	0.39	0.34	0.43	0.38	0.39	0.39	0.48	0.41	0.44	0.41	0.36	0.31
75 Sikha 8	0.56	0.58	0.45	0.56	0.56	0.49	0.84	0.83	0.54	0.54	0.56	0.58	0.73	0.47	0.47	0.56	0.61	0.38	0.47	0.44	0.47	0.49	0.47	0.45	0.48	0.34	0.42	0.38
76 Sipche 2	0.48	0.52	0.45	0.50	0.55	0.48	0.49	0.50	0.64	0.64	0.65	0.61	0.59	0.50	0.57	0.52	0.55	0.39	0.57	0.47	0.52	0.45	0.64	0.53	0.55	0.50	0.49	0.36
77 Sipche 3	0.55	0.52	0.43	0.48	0.55	0.48	0.45	0.50	0.64	0.61	0.61	0.57	0.54	0.52	0.52	0.55	0.57	0.43	0.59	0.45	0.52	0.48	0.66	0.60	0.60	0.45	0.45	0.34
78 Sipche 4	0.55	0.52	0.43	0.48	0.55	0.48	0.45	0.50	0.64	0.61	0.61	0.57	0.54	0.52	0.52	0.55	0.57	0.43	0.59	0.45	0.52	0.48	0.64	0.57	0.57	0.48	0.47	0.34
79 Sipche 6	0.55	0.52	0.43	0.48	0.55	0.48	0.45	0.50	0.64	0.61	0.61	0.57	0.54	0.52	0.52	0.55	0.57	0.43	0.59	0.45	0.52	0.48	0.64	0.57	0.57	0.48	0.47	0.34
80 Sipche 7	0.55	0.52	0.43	0.48	0.55	0.48	0.45	0.50	0.64	0.61	0.61	0.57	0.54	0.52	0.52	0.55	0.57	0.43	0.59	0.45	0.52	0.48	0.64	0.57	0.57	0.48	0.47	0.34
81 Sipche 9	0.55	0.52	0.43	0.48	0.55	0.48	0.45	0.50	0.64	0.61	0.61	0.57	0.54	0.52	0.52	0.55	0.57	0.43	0.59	0.45	0.52	0.48	0.64	0.57	0.57	0.48	0.47	0.34
82 Sipche 11	0.54	0.52	0.43	0.47	0.54	0.47	0.44	0.49	0.63	0.61	0.60	0.56	0.54	0.52	0.52	0.54	0.56	0.43	0.58	0.44	0.52	0.47	0.63	0.57	0.57	0.47	0.47	0.33
83 Sipche 12	0.47	0.47	0.34	0.45	0.47	0.45	0.40	0.45	0.54	0.52	0.51	0.49	0.52	0.34	0.43	0.38	0.43	0.43	0.58	0.38	0.38	0.43	0.61	0.55	0.55	0.52	0.40	0.33
84 Thangja 1	0.43	0.43	0.48	0.39	0.36	0.43	0.40	0.39	0.41	0.45	0.43	0.43	0.41	0.45	0.43	0.41	0.43	0.55	0.39	0.58	0.41	0.43	0.43	0.55	0.53	0.64	0.52	0.47
85 Thangja 2	0.50	0.50	0.50	0.37	0.39	0.48	0.43	0.43	0.48	0.45	0.45	0.45	0.39	0.52	0.52	0.50	0.55	0.59	0.43	0.52	0.48	0.52	0.36	0.41	0.41	0.66	0.54	0.43
86 Thangja 3	0.52	0.52	0.45	0.45	0.43	0.43	0.42	0.45	0.45	0.45	0.44	0.45	0.43	0.49	0.47	0.47	0.47	0.56	0.49	0.47	0.49	0.49	0.38	0.43	0.43	0.49	0.51	0.40
87 Thomje 2	0.40	0.40	0.27	0.34	0.34	0.34	0.36	0.38	0.40	0.40	0.40	0.40	0.41	0.31	0.36	0.29	0.31	0.38	0.47	0.33	0.31	0.38	0.47	0.45	0.45	0.47	0.29	0.29
88 Thomje 3	0.45	0.45	0.31	0.45	0.43	0.40	0.40	0.38	0.47	0.52	0.51	0.49	0.52	0.34	0.43	0.34	0.36	0.45	0.49	0.31	0.31	0.36	0.54	0.57	0.55	0.38	0.27	0.24
89 Thomje 4	0.48	0.50	0.36	0.50	0.45	0.43	0.43	0.41	0.50	0.55	0.56	0.52	0.54	0.32	0.43	0.41	0.41	0.41	0.59	0.40	0.34	0.41	0.68	0.64	0.62	0.45	0.36	0.31
90 Thomje 6	0.54	0.56	0.43	0.49	0.45	0.56	0.47	0.47	0.54	0.54	0.56	0.52	0.56	0.47	0.47	0.58	0.58	0.47	0.54	0.40	0.40	0.54	0.40	0.43	0.41	0.38	0.44	0.42
91 Thomje 7	0.48	0.48	0.36	0.50	0.48	0.45	0.43	0.43	0.52	0.55	0.56	0.52	0.54	0.32	0.43	0.41	0.43	0.45	0.64	0.40	0.34	0.43	0.66	0.62	0.62	0.45	0.36	0.31
92 Thonje 3	0.45	0.45	0.59	0.41	0.36	0.48	0.40	0.39	0.45	0.50	0.47	0.48	0.39	0.45	0.43	0.45	0.45	0.57	0.39	0.67	0.43	0.43	0.45	0.53	0.51	0.64	0.49	0.43
93 Thonje 4	0.52	0.52	0.48	0.43	0.43	0.48	0.40	0.43	0.52	0.52	0.54	0.52	0.46	0.41	0.52	0.52	0.52	0.59	0.50	0.52	0.50	0.50	0.48	0.51	0.51	0.55	0.52	0.43
94 Thonje 5	0.41	0.41	0.52	0.46	0.45	0.50	0.40	0.43	0.45	0.45	0.47	0.45	0.39	0.48	0.45	0.55	0.52	0.52	0.50	0.54	0.55	0.48	0.45	0.46	0.44	0.61	0.56	0.52
95 Thonje 6	0.57	0.55	0.55	0.44	0.46	0.48	0.43	0.44	0.37	0.34	0.34	0.37	0.33	0.57	0.44	0.55	0.55	0.60	0.44	0.55	0.60	0.60	0.41	0.44	0.44	0.55	0.55	0.48
96 Thonje 16	0.59	0.59	0.52	0.37	0.39	0.45	0.38	0.39	0.48	0.45	0.43	0.43	0.35	0.50	0.50	0.50	0.48	0.68	0.50	0.58	0.52	0.61	0.48	0.46	0.46	0.61	0.54	0.43
97 Thonje 18	0.66	0.61	0.61	0.41	0.43	0.57	0.49	0.45	0.45	0.48	0.47	0.50	0.46	0.52	0.43	0.64	0.66	0.59	0.50	0.58	0.45	0.64	0.34	0.44	0.41	0.50	0.52	0.47
98 Thonje 19	0.50	0.48	0.59	0.41	0.48	0.61	0.43	0.45	0.50	0.45	0.45	0.45	0.39	0.43	0.41	0.52	0.57	0.48	0.43	0.61	0.45	0.52	0.41	0.44	0.41	0.59	0.49	0.43
99 Thonje 21	0.67	0.70	0.45	0.37	0.43	0.52	0.53	0.56	0.52	0.49	0.51	0.54	0.54	0.47	0.49	0.52	0.56	0.52	0.47	0.51	0.45	0.65	0.36	0.36	0.39	0.40	0.42	0.38
100 Tilman camp 1	0.43	0.43	0.57	0.43	0.41	0.48	0.38	0.39	0.45	0.43	0.40	0.41	0.33	0.45	0.43	0.45	0.45	0.52	0.41	0.65	0.45	0.45	0.43	0.46	0.46	0.68	0.54	0.47

Appendix-I Cont.

	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
101 Tilman camp 8	0.48	0.48	0.50	0.37	0.39	0.50	0.43	0.43	0.48	0.45	0.45	0.45	0.39	0.50	0.52	0.52	0.55	0.59	0.43	0.49	0.43	0.50	0.36	0.41	0.41	0.61	0.54	0.45
102 Tsumje 1	0.63	0.70	0.56	0.45	0.45	0.52	0.60	0.54	0.49	0.52	0.53	0.49	0.54	0.38	0.40	0.54	0.47	0.52	0.56	0.56	0.47	0.58	0.38	0.41	0.41	0.40	0.40	0.36
103 Tsumje 2	0.56	0.58	0.47	0.56	0.56	0.49	0.49	0.49	0.52	0.49	0.49	0.47	0.52	0.58	0.58	0.63	0.56	0.54	0.65	0.49	0.56	0.63	0.49	0.48	0.48	0.45	0.53	0.51
104 Tukucha	0.85	0.81	0.52	0.45	0.49	0.56	0.58	0.56	0.52	0.52	0.53	0.56	0.56	0.52	0.47	0.58	0.61	0.65	0.61	0.58	0.49	0.83	0.38	0.43	0.45	0.40	0.42	0.44
105 Ulleri 9	0.67	0.67	0.49	0.47	0.45	0.49	0.69	0.65	0.49	0.52	0.53	0.56	0.60	0.36	0.40	0.45	0.52	0.47	0.49	0.49	0.43	0.61	0.38	0.43	0.43	0.43	0.44	0.40
106 Ulleri 21	0.66	0.66	0.50	0.48	0.45	0.50	0.72	0.68	0.48	0.50	0.52	0.55	0.61	0.39	0.39	0.43	0.50	0.45	0.48	0.49	0.43	0.59	0.41	0.46	0.46	0.43	0.43	0.38
107 Solu Uwa	0.48	0.52	0.45	0.57	0.55	0.43	0.61	0.55	0.45	0.48	0.49	0.50	0.54	0.36	0.39	0.36	0.41	0.34	0.45	0.43	0.41	0.41	0.39	0.46	0.48	0.43	0.38	0.38

(Continued in next pages)

Appendix-I Cont.

	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84
58 Pisang 8	0.76																											
59 Pisang 9	0.72	0.72																										
60 Pork 1	0.40	0.49	0.48																									
61 Pork 2	0.38	0.40	0.45	0.76																								
62 Sama 1	0.31	0.31	0.38	0.36	0.44																							
63 Sama 2	0.24	0.29	0.38	0.34	0.36	0.73																						
64 Sama 3	0.34	0.40	0.43	0.43	0.36	0.40	0.54																					
65 Sama 4	0.34	0.38	0.39	0.43	0.36	0.54	0.61	0.55																				
66 Sama 6	0.24	0.27	0.36	0.36	0.38	0.73	0.93	0.56	0.58																			
67 Sama 8	0.45	0.56	0.55	0.43	0.38	0.27	0.34	0.73	0.48	0.34																		
68 Sama 9	0.47	0.54	0.55	0.50	0.47	0.29	0.31	0.59	0.41	0.34	0.77																	
69 Sikha 1	0.34	0.38	0.41	0.39	0.43	0.47	0.40	0.43	0.50	0.43	0.45	0.41																
70 Sikha 2	0.34	0.38	0.41	0.39	0.40	0.47	0.43	0.45	0.52	0.47	0.48	0.39	0.93															
71 Sikha 4	0.38	0.44	0.43	0.43	0.42	0.40	0.40	0.40	0.43	0.44	0.45	0.40	0.70	0.74														
72 Sikha 5	0.34	0.38	0.48	0.39	0.45	0.49	0.40	0.41	0.45	0.40	0.41	0.34	0.91	0.89	0.67													
73 Sikha 6	0.34	0.38	0.41	0.41	0.40	0.49	0.45	0.48	0.50	0.49	0.45	0.36	0.91	0.95	0.79	0.89												
74 Sikha 7	0.34	0.38	0.41	0.34	0.36	0.49	0.47	0.43	0.48	0.49	0.43	0.34	0.84	0.89	0.76	0.84	0.93											
75 Sikha 8	0.42	0.47	0.45	0.45	0.42	0.42	0.36	0.38	0.47	0.40	0.45	0.40	0.70	0.74	0.89	0.70	0.76	0.74										
76 Sipche 2	0.40	0.45	0.45	0.45	0.45	0.56	0.47	0.45	0.61	0.49	0.45	0.45	0.50	0.52	0.54	0.55	0.57	0.57	0.54									
77 Sipche 3	0.38	0.45	0.43	0.43	0.45	0.52	0.43	0.45	0.59	0.43	0.50	0.45	0.48	0.50	0.52	0.50	0.52	0.52	0.52	0.84								
78 Sipche 4	0.38	0.45	0.45	0.43	0.45	0.52	0.40	0.43	0.57	0.40	0.48	0.45	0.48	0.48	0.49	0.52	0.50	0.50	0.49	0.86	0.98							
79 Sipche 6	0.38	0.45	0.45	0.43	0.45	0.52	0.40	0.43	0.57	0.40	0.48	0.45	0.48	0.48	0.49	0.52	0.50	0.50	0.49	0.86	0.98	1.00						
80 Sipche 7	0.38	0.45	0.45	0.43	0.45	0.52	0.40	0.43	0.57	0.40	0.48	0.45	0.48	0.48	0.49	0.52	0.50	0.50	0.49	0.86	0.98	1.00	1.00					
81 Sipche 9	0.38	0.45	0.45	0.43	0.45	0.52	0.40	0.43	0.57	0.40	0.48	0.45	0.48	0.48	0.49	0.52	0.50	0.50	0.49	0.86	0.98	1.00	1.00	1.00				
82 Sipche 11	0.38	0.44	0.45	0.43	0.47	0.51	0.40	0.43	0.56	0.40	0.47	0.45	0.47	0.47	0.49	0.52	0.49	0.49	0.49	0.85	0.97	0.99	0.99	0.99	0.99			
83 Sipche 12	0.38	0.38	0.45	0.36	0.42	0.78	0.73	0.45	0.61	0.73	0.36	0.36	0.47	0.47	0.42	0.52	0.49	0.49	0.44	0.67	0.61	0.63	0.63	0.63	0.63	0.62		
84 Thangja 1	0.45	0.52	0.52	0.48	0.40	0.31	0.40	0.52	0.43	0.43	0.57	0.57	0.39	0.41	0.45	0.36	0.43	0.41	0.43	0.43	0.43	0.41	0.41	0.41	0.41	0.40	0.43	
85 Thangja 2	0.47	0.56	0.52	0.50	0.47	0.34	0.31	0.43	0.39	0.31	0.50	0.52	0.34	0.34	0.38	0.36	0.32	0.32	0.38	0.48	0.43	0.45	0.45	0.45	0.45	0.45	0.38	0.59
86 Thangja 3	0.42	0.51	0.49	0.47	0.51	0.40	0.38	0.45	0.38	0.40	0.45	0.49	0.38	0.38	0.47	0.43	0.40	0.40	0.44	0.56	0.52	0.54	0.54	0.54	0.54	0.53	0.49	0.58
87 Thomje 2	0.27	0.29	0.36	0.34	0.38	0.73	0.82	0.52	0.56	0.84	0.29	0.34	0.40	0.40	0.38	0.43	0.43	0.45	0.33	0.52	0.45	0.45	0.45	0.45	0.45	0.44	0.76	0.38
88 Thomje 3	0.27	0.29	0.34	0.40	0.40	0.73	0.76	0.52	0.72	0.78	0.34	0.31	0.47	0.49	0.44	0.45	0.52	0.49	0.44	0.58	0.54	0.52	0.52	0.52	0.52	0.51	0.78	0.47
89 Thomje 4	0.36	0.38	0.43	0.41	0.38	0.70	0.72	0.50	0.70	0.72	0.43	0.39	0.50	0.52	0.45	0.48	0.55	0.52	0.47	0.68	0.64	0.61	0.61	0.61	0.61	0.61	0.85	0.48
90 Thomje 6	0.42	0.47	0.52	0.49	0.47	0.58	0.53	0.45	0.54	0.53	0.45	0.40	0.40	0.43	0.47	0.40	0.43	0.36	0.51	0.54	0.49	0.47	0.47	0.47	0.47	0.47	0.58	0.45
91 Thomje 7	0.38	0.36	0.43	0.39	0.38	0.70	0.67	0.48	0.68	0.70	0.41	0.39	0.50	0.52	0.45	0.48	0.55	0.52	0.47	0.68	0.66	0.64	0.64	0.64	0.64	0.63	0.85	0.45
92 Thonje 3	0.45	0.58	0.55	0.50	0.40	0.31	0.45	0.66	0.48	0.47	0.68	0.64	0.39	0.41	0.45	0.36	0.43	0.41	0.43	0.50	0.48	0.45	0.45	0.45	0.45	0.45	0.45	0.80
93 Thonje 4	0.47	0.56	0.52	0.48	0.45	0.40	0.38	0.50	0.50	0.40	0.48	0.52	0.39	0.39	0.43	0.43	0.41	0.41	0.45	0.57	0.55	0.57	0.57	0.57	0.57	0.56	0.52	0.64
94 Thonje 5	0.52	0.61	0.59	0.50	0.43	0.45	0.40	0.48	0.45	0.36	0.55	0.61	0.43	0.43	0.43	0.45	0.45	0.45	0.40	0.48	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.68

Appendix-I Cont.

	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84
95 Thonje 6	0.55	0.61	0.57	0.48	0.43	0.32	0.30	0.46	0.44	0.30	0.55	0.51	0.37	0.37	0.41	0.39	0.34	0.34	0.41	0.44	0.48	0.51	0.51	0.51	0.51	0.50	0.39	0.57
96 Thonje 16	0.47	0.58	0.52	0.52	0.49	0.40	0.36	0.45	0.43	0.36	0.52	0.57	0.34	0.34	0.36	0.36	0.32	0.32	0.36	0.52	0.50	0.52	0.52	0.52	0.52	0.52	0.45	0.55
97 Thonje 18	0.54	0.63	0.61	0.50	0.43	0.29	0.31	0.48	0.45	0.34	0.57	0.55	0.43	0.43	0.47	0.39	0.41	0.39	0.49	0.45	0.48	0.48	0.48	0.48	0.48	0.47	0.38	0.52
98 Thonje 19	0.52	0.58	0.52	0.36	0.34	0.36	0.36	0.50	0.43	0.36	0.61	0.57	0.41	0.41	0.43	0.41	0.39	0.39	0.45	0.52	0.50	0.50	0.50	0.50	0.50	0.49	0.49	0.52
99 Thonje 21	0.38	0.44	0.49	0.49	0.49	0.40	0.42	0.38	0.43	0.40	0.40	0.45	0.45	0.45	0.56	0.47	0.47	0.49	0.58	0.49	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.40
100 Tilman camp 1	0.52	0.58	0.59	0.41	0.40	0.34	0.40	0.55	0.45	0.43	0.61	0.61	0.41	0.41	0.36	0.43	0.39	0.39	0.36	0.50	0.43	0.45	0.45	0.45	0.45	0.45	0.49	0.73
101 Tilman camp 8	0.47	0.56	0.52	0.50	0.47	0.34	0.36	0.45	0.41	0.36	0.50	0.52	0.34	0.34	0.38	0.36	0.32	0.32	0.38	0.50	0.45	0.48	0.48	0.48	0.48	0.47	0.43	0.61
102 Tsumje 1	0.38	0.44	0.47	0.47	0.51	0.42	0.38	0.43	0.47	0.42	0.54	0.52	0.56	0.58	0.58	0.52	0.56	0.49	0.58	0.52	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.43
103 Tsumje 2	0.49	0.53	0.54	0.63	0.67	0.40	0.36	0.36	0.40	0.36	0.45	0.52	0.47	0.47	0.51	0.47	0.45	0.40	0.51	0.52	0.54	0.54	0.54	0.54	0.54	0.53	0.47	0.43
104 Tukucha	0.51	0.51	0.54	0.54	0.49	0.38	0.36	0.38	0.45	0.38	0.47	0.52	0.45	0.45	0.58	0.40	0.45	0.43	0.60	0.47	0.52	0.52	0.52	0.52	0.52	0.51	0.44	0.40
105 Ulleri 9	0.40	0.49	0.49	0.47	0.42	0.42	0.42	0.40	0.49	0.42	0.49	0.43	0.61	0.61	0.73	0.58	0.61	0.58	0.73	0.49	0.45	0.47	0.47	0.47	0.47	0.47	0.49	0.47
106 Ulleri 21	0.38	0.47	0.48	0.45	0.38	0.43	0.43	0.43	0.52	0.43	0.50	0.43	0.64	0.64	0.74	0.61	0.64	0.61	0.74	0.52	0.48	0.50	0.50	0.50	0.50	0.49	0.49	0.48
107 Solu Uwa	0.36	0.36	0.41	0.41	0.36	0.38	0.40	0.41	0.50	0.45	0.45	0.34	0.68	0.70	0.61	0.66	0.70	0.66	0.58	0.55	0.45	0.48	0.48	0.48	0.48	0.47	0.43	0.41

	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106
86 Thangja 3	0.65																					
87 Thomje 2	0.36	0.42																				
88 Thomje 3	0.34	0.47	0.78																			
89 Thomje 4	0.34	0.40	0.70	0.81																		
90 Thomje 6	0.43	0.44	0.53	0.71	0.65																	
91 Thomje 7	0.34	0.40	0.67	0.76	0.95	0.61																
92 Thonje 3	0.59	0.65	0.40	0.49	0.50	0.47	0.48															
93 Thonje 4	0.59	0.70	0.40	0.49	0.48	0.45	0.48	0.68														
94 Thonje 5	0.57	0.63	0.40	0.38	0.43	0.47	0.41	0.66	0.66													
95 Thonje 6	0.62	0.66	0.32	0.36	0.32	0.45	0.32	0.62	0.64	0.67												
96 Thonje 16	0.70	0.61	0.38	0.36	0.39	0.45	0.39	0.57	0.66	0.64	0.71											
97 Thonje 18	0.61	0.52	0.34	0.36	0.41	0.52	0.39	0.57	0.55	0.52	0.60	0.57										
98 Thonje 19	0.55	0.45	0.40	0.38	0.45	0.49	0.45	0.66	0.52	0.57	0.53	0.52	0.66									
99 Thonje 21	0.47	0.51	0.42	0.42	0.45	0.49	0.45	0.40	0.49	0.43	0.45	0.49	0.56	0.47								
100 Tilman camp 1	0.61	0.63	0.43	0.43	0.43	0.38	0.43	0.86	0.73	0.64	0.67	0.59	0.55	0.66	0.38							
101 Tilman camp 8	0.91	0.67	0.36	0.38	0.36	0.43	0.36	0.66	0.66	0.59	0.64	0.70	0.64	0.57	0.43	0.68						
102 Tsumje 1	0.45	0.49	0.40	0.44	0.49	0.60	0.49	0.47	0.49	0.47	0.50	0.52	0.52	0.47	0.60	0.45	0.45					
103 Tsumje 2	0.54	0.58	0.40	0.40	0.43	0.53	0.43	0.45	0.47	0.52	0.55	0.56	0.58	0.47	0.49	0.47	0.58	0.58				
104 Tukucha	0.47	0.51	0.38	0.40	0.43	0.51	0.47	0.43	0.49	0.40	0.52	0.56	0.67	0.52	0.76	0.40	0.45	0.64	0.58			
105 Ulleri 9	0.49	0.51	0.40	0.47	0.49	0.58	0.47	0.45	0.52	0.43	0.52	0.49	0.56	0.40	0.62	0.43	0.49	0.69	0.53	0.69		
106 Ulleri 21	0.48	0.47	0.40	0.47	0.52	0.54	0.50	0.45	0.50	0.43	0.53	0.48	0.55	0.41	0.58	0.43	0.48	0.65	0.49	0.65	0.97	
107 Solu Uwa	0.41	0.43	0.40	0.43	0.48	0.45	0.48	0.36	0.39	0.41	0.44	0.36	0.43	0.39	0.43	0.41	0.41	0.54	0.45	0.47	0.67	0.66

Appendix-II UPGMA clustering groups of the Nepalese hulless barley landraces

Group	Landraces	Group	Landraces	Group	Landraces
(I)	Annapurna BC-1		Sipche-7		Sikha-6
	Annapurna BC-2		Sipche-9		Sikha-5
	Thangja-1		Sipche-11		Sikha-7
	Thonje-3		Nepal-3		Solu Uwa
	Tilman Camp-1		Nepal-4		
	Bimtakothi-1		Nepal-5	(VIII)	N-6
	Bimtakothi-5		Nepal-6		N-12
	Bimtakothi-10		Nepal-7		
	Bimtakothi-2			(IX)	Philem-1
	Bimtakothi-4	(V)	Gho-1		Philem-2
	Bimtakothi-12		Gho-2		Philem-3
	Thonje-5		Ngyak-4		
	Thonje-6		Ngyak-3	(X)	Sama-1
	Chame-2		Ngyak-1		Sama-2
	Pisang-4		Ngyak-10		Sama-6
	Thangja-3		Tsumje-2		Thomje-2
	Thonje-4		Gho-3		Sipche-12
	Bimtakothi-13		Ngyak-12		Thomje-4
	Chame-3		Ngyak-2		Thomje-7
	Thonje-16		Pork-2		Thomje-3
	Tilman Camp-7		Pork-1		Sama-4
	Chame-11				Thomje-6
	Chame-13	(VI)	Chame-9		
	Chame-12		Dhumpu-2		
	Thangja-2		Jomson-1		
	Tilman Camp-8		Jomson-2		
			Kagbeni-3		
(II)	Lih Dhanra Gal		Kagbeni-5		
	Sama-8		Tukucha		
	Ngyak-11		Phalatey		
	Sama-9		Thonje-21		
	Sama-3		Tsumje-1		
			Chame-14		
(III)	Pisang-5		Thonje-18		
	Pisang-6		Thonje-19		
	Pisang-7		Naked-304		
	Pisang-8				
	Pisang-9	(VII)	Ghara-1		
			Ghara-2		
(IV)	Bimtakothi-3		Nepal-1		
	Bimtakothi-9		Nepal-2		
	Bimtakothi-11		Sikha-4		
	Chame-8		Sikha-8		
	Sipche-2		Ulleri-9		
	Sipche-3		Ulleri-21		
	Sipche-4		Sikha-1		
	Sipche-6		Sikha-2		

Genotypes are in original order of the dendrogram (Fig. 8) from the top (left) to the bottom (right)

Appendix-III Group membership probability (shared ancestry): (1)=Sikha, (2)=Nepal, (3)=Ngyak, (4)=Sama, (5)=Jomson, (6)=Pisang, (7)=Thonje, (8)=Bimtakothi, (9)=Sipche

Landraces	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Annapurna BC-1	0.002	0.002	0.010	0.009	0.002	0.015	0.542	0.415	0.001
Annapurna BC-2	0.001	0.002	0.003	0.009	0.002	0.006	0.400	0.574	0.002
Bimtakothi-1	0.002	0.002	0.003	0.002	0.002	0.003	0.436	0.548	0.002
Bimtakothi-2	0.002	0.002	0.018	0.004	0.004	0.004	0.535	0.430	0.002
Bimtakothi-3	0.005	0.178	0.003	0.002	0.002	0.003	0.003	0.801	0.004
Bimtakothi-4	0.002	0.002	0.003	0.002	0.002	0.002	0.555	0.430	0.002
Bimtakothi-5	0.002	0.002	0.003	0.004	0.002	0.005	0.545	0.434	0.002
Bimtakothi-9	0.012	0.145	0.004	0.002	0.002	0.005	0.004	0.819	0.009
Bimtakothi-10	0.002	0.005	0.003	0.006	0.004	0.016	0.539	0.421	0.003
Bimtakothi-11	0.004	0.199	0.003	0.002	0.002	0.004	0.003	0.779	0.004
Bimtakothi-12	0.004	0.007	0.077	0.009	0.007	0.015	0.465	0.413	0.002
Bimtakothi-13	0.002	0.007	0.005	0.009	0.016	0.007	0.547	0.402	0.004
Chame-2	0.003	0.002	0.012	0.008	0.023	0.004	0.526	0.419	0.002
Chame-3	0.002	0.005	0.007	0.005	0.018	0.003	0.555	0.400	0.006
Chame-8	0.004	0.016	0.111	0.005	0.608	0.053	0.031	0.146	0.027
Chame-9	0.002	0.004	0.005	0.002	0.978	0.004	0.002	0.002	0.002
Chame-11	0.004	0.018	0.007	0.008	0.046	0.010	0.504	0.403	0.002
Chame-12	0.002	0.011	0.011	0.007	0.007	0.004	0.583	0.373	0.002
Chame-13	0.002	0.002	0.004	0.003	0.005	0.004	0.553	0.426	0.002
Chame-14	0.003	0.003	0.005	0.005	0.801	0.010	0.072	0.097	0.003
Dhumpu-2	0.004	0.031	0.004	0.004	0.945	0.005	0.002	0.002	0.002
Ghara-1	0.026	0.922	0.003	0.003	0.009	0.006	0.003	0.012	0.016
Ghara-2	0.030	0.942	0.013	0.002	0.003	0.002	0.002	0.003	0.002
Gho-1	0.002	0.004	0.969	0.002	0.011	0.003	0.003	0.003	0.003
Gho-2	0.004	0.010	0.829	0.009	0.070	0.048	0.012	0.014	0.002
Gho-3	0.002	0.004	0.937	0.004	0.006	0.003	0.020	0.014	0.010
Jomson-1	0.002	0.002	0.083	0.002	0.898	0.004	0.004	0.002	0.002
Jomson-2	0.002	0.002	0.003	0.002	0.983	0.002	0.002	0.002	0.002
Kagbeni-3	0.002	0.003	0.002	0.002	0.982	0.002	0.002	0.002	0.002
Kagbeni-5	0.002	0.008	0.009	0.006	0.966	0.003	0.002	0.002	0.002
Lih Dhanra Gal	0.002	0.024	0.003	0.936	0.013	0.006	0.006	0.008	0.001
N-6	0.005	0.869	0.018	0.047	0.013	0.012	0.008	0.025	0.003
N-12	0.013	0.812	0.025	0.026	0.018	0.014	0.007	0.082	0.004
Naked-304	0.006	0.117	0.013	0.134	0.583	0.082	0.004	0.058	0.003
Nepal-1	0.017	0.947	0.003	0.004	0.020	0.003	0.002	0.003	0.001
Nepal-2	0.010	0.968	0.004	0.002	0.007	0.003	0.002	0.003	0.002
Nepal-3	0.002	0.975	0.004	0.003	0.003	0.003	0.002	0.003	0.004
Nepal-4	0.002	0.980	0.002	0.003	0.003	0.002	0.002	0.002	0.004
Nepal-5	0.002	0.976	0.005	0.003	0.003	0.002	0.002	0.003	0.004
Neapl-6	0.003	0.967	0.003	0.009	0.004	0.003	0.004	0.005	0.003

Appendix-III Cont.

Landraces	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Nepal-7	0.016	0.959	0.012	0.002	0.004	0.002	0.002	0.002	0.002
Ngyak-1	0.002	0.007	0.970	0.002	0.004	0.004	0.003	0.005	0.002
Ngyak-2	0.002	0.006	0.977	0.002	0.003	0.002	0.002	0.003	0.002
Ngyak-3	0.003	0.020	0.923	0.003	0.019	0.018	0.003	0.009	0.002
Ngyak-4	0.005	0.012	0.935	0.003	0.014	0.011	0.005	0.013	0.002
Tilman camp-7	0.002	0.002	0.004	0.004	0.291	0.002	0.406	0.286	0.002
Ngyak-10	0.004	0.207	0.568	0.003	0.030	0.027	0.004	0.051	0.105
Ngyak-11	0.002	0.011	0.003	0.956	0.004	0.003	0.011	0.007	0.002
Ngyak-12	0.003	0.005	0.760	0.010	0.004	0.003	0.100	0.111	0.003
Phalatey	0.002	0.007	0.004	0.004	0.963	0.009	0.003	0.008	0.002
Philem-1	0.003	0.005	0.692	0.009	0.003	0.004	0.012	0.007	0.265
Philem-2	0.005	0.004	0.935	0.007	0.003	0.006	0.012	0.007	0.021
Philem-3	0.004	0.005	0.949	0.004	0.002	0.016	0.004	0.003	0.013
Pisang-4	0.003	0.004	0.012	0.083	0.002	0.014	0.516	0.362	0.003
Pisang-5	0.002	0.002	0.005	0.006	0.004	0.956	0.005	0.018	0.002
Pisang-6	0.002	0.009	0.010	0.004	0.011	0.959	0.002	0.003	0.001
Pisang-7	0.002	0.002	0.002	0.003	0.002	0.982	0.002	0.003	0.001
Pisang-8	0.002	0.002	0.003	0.005	0.004	0.969	0.010	0.003	0.002
Pisang-9	0.006	0.003	0.003	0.005	0.007	0.953	0.009	0.006	0.010
Pork-1	0.017	0.003	0.838	0.033	0.011	0.076	0.017	0.002	0.002
Pork-2	0.005	0.013	0.890	0.058	0.015	0.009	0.004	0.003	0.004
Sama-1	0.005	0.004	0.011	0.003	0.002	0.004	0.005	0.005	0.962
Sama-2	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.984
Sama-3	0.003	0.002	0.002	0.602	0.002	0.002	0.005	0.003	0.381
Sama-4	0.032	0.020	0.016	0.031	0.013	0.014	0.010	0.014	0.851
Sama-6	0.010	0.002	0.002	0.002	0.002	0.001	0.002	0.002	0.978
Sama-8	0.003	0.002	0.002	0.977	0.002	0.003	0.006	0.002	0.003
Sama-9	0.003	0.002	0.005	0.963	0.003	0.008	0.012	0.002	0.002
Sikha-1	0.983	0.003	0.002	0.004	0.001	0.002	0.001	0.002	0.002
Sikha-2	0.987	0.002	0.001	0.002	0.001	0.001	0.001	0.002	0.002
Sikha-4	0.980	0.004	0.002	0.002	0.003	0.002	0.002	0.002	0.002
Sikha-5	0.979	0.003	0.003	0.002	0.002	0.003	0.002	0.003	0.003
Sikha-6	0.987	0.002	0.001	0.001	0.001	0.001	0.001	0.002	0.002
Sikha-7	0.976	0.004	0.001	0.002	0.001	0.002	0.001	0.002	0.010
Sikha-8	0.957	0.015	0.005	0.002	0.008	0.003	0.002	0.004	0.004
Sipche-2	0.076	0.004	0.003	0.003	0.004	0.006	0.008	0.006	0.888
Sipche-3	0.003	0.004	0.003	0.002	0.003	0.002	0.002	0.004	0.978
Sipche-4	0.003	0.004	0.003	0.002	0.003	0.002	0.002	0.004	0.978
Sipche-6	0.003	0.004	0.003	0.002	0.003	0.002	0.002	0.004	0.978
Sipche-7	0.003	0.004	0.003	0.002	0.003	0.002	0.002	0.004	0.978
Sipche-9	0.003	0.004	0.003	0.002	0.003	0.002	0.003	0.004	0.977
Sipche-11	0.003	0.004	0.003	0.002	0.003	0.002	0.002	0.004	0.977

Appendix-III Cont.

Landraces	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Sipche-12	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.984
Thangja-1	0.014	0.004	0.003	0.018	0.002	0.004	0.939	0.007	0.009
Thangja-2	0.002	0.002	0.003	0.004	0.006	0.004	0.974	0.003	0.002
Thangja-3	0.004	0.004	0.004	0.004	0.009	0.003	0.954	0.006	0.014
Thomje-2	0.003	0.002	0.006	0.006	0.011	0.002	0.006	0.002	0.963
Thomje-3	0.002	0.002	0.004	0.002	0.002	0.002	0.009	0.003	0.974
Thomje-4	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.986
Thomje-6	0.009	0.007	0.386	0.004	0.009	0.014	0.005	0.012	0.554
Thomje-7	0.002	0.002	0.002	0.002	0.004	0.003	0.002	0.002	0.980
Thonje-3	0.003	0.002	0.002	0.046	0.002	0.003	0.934	0.004	0.005
Thonje-4	0.004	0.009	0.006	0.007	0.010	0.004	0.945	0.007	0.008
Thonje-5	0.003	0.002	0.005	0.013	0.003	0.006	0.908	0.055	0.004
Thonje-6	0.002	0.002	0.004	0.006	0.004	0.006	0.920	0.046	0.009
Thonje-16	0.002	0.002	0.003	0.003	0.004	0.002	0.927	0.053	0.005
Thonje-18	0.004	0.004	0.010	0.024	0.677	0.034	0.218	0.027	0.002
Thonje-19	0.003	0.004	0.006	0.243	0.330	0.050	0.327	0.018	0.020
Thonje-21	0.016	0.003	0.003	0.004	0.953	0.011	0.002	0.003	0.005
Tilman camp-1	0.002	0.002	0.002	0.013	0.002	0.004	0.967	0.008	0.002
Tilman camp-8	0.002	0.002	0.002	0.002	0.002	0.002	0.983	0.003	0.002
Tsumje-1	0.527	0.020	0.024	0.152	0.164	0.016	0.060	0.010	0.027
Tsumje-2	0.083	0.009	0.803	0.013	0.059	0.009	0.011	0.008	0.004
Tukucha	0.003	0.002	0.002	0.003	0.977	0.005	0.002	0.002	0.004
Ulleri-9	0.951	0.004	0.003	0.004	0.023	0.004	0.005	0.003	0.003
Ulleri-21	0.966	0.003	0.002	0.003	0.012	0.003	0.004	0.003	0.003
Solu Uwa	0.811	0.018	0.089	0.021	0.004	0.009	0.004	0.008	0.037

Appendix-IV Model based groups of the Nepalese hulless barley landraces

Group	Landraces	Group	Landraces	Group	Landraces
(1)	Sikha-2	(5)	Jomson-2		Bimtakothi-12
	Sikha-6		Kagbeni-3		Tilman camp-7
	Sikha-1		Chame-9		Bimtakothi-9
	Sikha-4		Tukucha		Bimtakothi-3
	Sikha-5		Kagbeni-5		Bimtakothi-11
	Sikha-7		Phalatey		Annapurna BC-2
	Ulleri-21		Thonje-21		Bimtakothi-1
	Sikha-8		Dhumpu-2		
	Ulleri-9		Jomson-1	(9)	Thomje-4
	Solu Uwa		Chame-14		Sama-2
	Tsumje-1		Thonje-18		Sipche-12
			Chame-8		Thomje-7
(2)	Nepal-4		Naked-304		Sama-6
	Nepal-5		Thonje-19		Sipche-3
	Nepal-3				Sipche-4
	Nepal-2				Sipche-6
	Neapl-6	(6)	Pisang-7		Sipche-7
	Nepal-7		Pisang-8		Sipche-9
	Nepal-1		Pisang-6		Sipche-11
	Ghara-2		Pisang-5		Thomje-3
	Ghara-1		Pisang-9		Thomje-2
	N-6				Sama-1
	N-12				Sipche-2
					Sama-4
(3)	Ngyak-2	(7)	Til.camp-8		Thomje-6
	Ngyak-1		Thangja-2		
	Gho-1		Til.camp-1		
	Philem-3		Thangja-3		
	Gho-3		Thonje-4		
	Ngyak-4		Thangja-1		
	Philem-2		Thonje-3		
	Ngyak-3		Thonje-16		
	Pork-2		Thonje-6		
	Pork-1		Thonje-5		
	Gho-2				
	Tsumje-2	(8)	Chame-12		
	Ngyak-12		Bimtakothi-4		
	Philem-1		Chame-3		
	Ngyak-10		Chame-13		
			Bimtakothi-13		
(4)	Sama-8		Bimtakothi-5		
	Sama-9		Annapurna BC-1		
	Ngyak-11		Bimtakothi-10		
	Lih D. Gal		Bimtakothi-2		
	Sama-3		Chame-2		
			Pisang-4		
			Chame-11		

Genotypes are in original order of the model based grouping (Fig. 9) from the top (left) to the bottom (right)

Appendix-V Allele frequency data

SSRs	Alleles (bp)	Frequency		
		Nepalese landraces (NL) (n=107)	German cultivars (GC) (n=35)	NL+GC (n=142)
Bmag0120	258	0.000	0.057	0.014
	237	0.047	0.000	0.035
	235	0.617	0.571	0.606
	233	0.047	0.000	0.035
	232	0.000	0.057	0.014
	230	0.290	0.114	0.246
	228	0.000	0.200	0.049
Bmag0136	204	0.009	0.000	0.007
	201	0.981	0.057	0.754
	200	0.009	0.943	0.239
Bmac0018	141	0.000	0.200	0.049
	139	0.084	0.514	0.190
	137	0.000	0.286	0.070
	135	0.636	0.000	0.479
	133	0.280	0.000	0.211
Bmag0218	194	0.150	0.000	0.113
	192	0.850	0.000	0.641
	188	0.000	1.000	0.246
Bmac0316	169	0.000	0.029	0.007
	162	0.991	0.029	0.754
	160	0.009	0.657	0.169
	135	0.000	0.029	0.007
	129	0.000	0.257	0.063
HVM40	162	0.000	0.229	0.056
	159	0.037	0.000	0.028
	157	0.636	0.000	0.479
	154	0.327	0.000	0.246
	148	0.000	0.114	0.028
	146	0.000	0.657	0.162
Bmac0093	160	0.000	0.114	0.028
	158	0.028	0.029	0.028
	156	0.327	0.771	0.437
	154	0.439	0.057	0.345
	151	0.196	0.029	0.155
	145	0.009	0.000	0.007
Bmag0353	125	0.000	0.029	0.007
	124	0.093	0.200	0.120

Appendix-V Cont.

SSRs	Alleles (bp)	Frequency		
		NL	GC	NL+GC
Bmag0353	121	0.187	0.257	0.204
	119	0.084	0.029	0.070
	118	0.028	0.086	0.042
	96	0.533	0.343	0.486
	94	0.000	0.057	0.014
	92	0.075	0.000	0.056
HvMLO3	233	0.299	0.771	0.415
	231	0.701	0.229	0.585
HVCMA	140	0.000	0.371	0.092
	132	0.776	0.000	0.585
	130	0.224	0.629	0.324
Bmac0209	193	0.000	0.171	0.042
	191	0.000	0.314	0.077
	189	0.000	0.229	0.056
	187	0.187	0.000	0.141
	185	0.729	0.000	0.549
	183	0.075	0.000	0.056
	182	0.009	0.000	0.007
	174	0.000	0.286	0.070
EBmac0684	187	0.009	0.000	0.007
	184	0.150	0.657	0.275
	182	0.037	0.286	0.099
	180	0.206	0.057	0.169
	179	0.019	0.000	0.014
	174	0.579	0.000	0.437
WMC1E8	233	0.963	0.543	0.859
	218	0.037	0.000	0.028
	188	0.000	0.457	0.113
HVLEU	168	0.000	0.257	0.063
	164	1.000	0.714	0.930
	162	0.000	0.029	0.007
HVM67	120	0.000	0.029	0.007
	119	0.000	0.029	0.007
	117	0.000	0.771	0.190
	115	0.785	0.000	0.592
	112	0.196	0.029	0.155
	111	0.009	0.143	0.042
	103	0.009	0.000	0.007

Appendix-V Cont.

SSRs	Alleles (bp)	Frequency		
		NL	GC	NL+GC
Bmag0009	180	0.000	0.029	0.007
	178	0.000	0.029	0.007
	176	0.112	0.171	0.127
	174	0.037	0.286	0.099
	173	0.028	0.057	0.035
	172	0.196	0.400	0.246
	171	0.626	0.000	0.472
EBmac0970	190	0.963	0.114	0.754
	188	0.037	0.800	0.225
	186	0.000	0.086	0.021
Bmac0399	144	0.000	0.029	0.007
	142	0.037	0.029	0.035
	140	0.308	0.171	0.275
	139	0.159	0.000	0.120
	138	0.009	0.629	0.162
	136	0.075	0.029	0.063
	130	0.000	0.029	0.007
	129	0.000	0.057	0.014
	127	0.000	0.029	0.007
	125	0.411	0.000	0.310
EBmac0415	248	0.262	0.029	0.204
	246	0.290	0.943	0.451
	237	0.458	0.000	0.345
	227	0.000	0.029	0.007
EBmac0701	154	0.000	0.057	0.014
	150	0.000	0.143	0.035
	147	0.000	0.629	0.155
	145	0.000	0.029	0.007
	141	0.000	0.143	0.035
	136	0.037	0.000	0.028
	134	0.682	0.000	0.514
	132	0.150	0.000	0.113
	119	0.131	0.000	0.099
Bmac0067	177	0.000	0.086	0.021
	175	0.150	0.486	0.232
	173	0.103	0.000	0.077
	170	0.411	0.400	0.408
	168	0.075	0.029	0.063

Appendix-V Cont.

SSRs	Alleles (bp)	Frequency		
		NL	GC	NL+GC
Bmac0067	156	0.047	0.000	0.035
	154	0.168	0.000	0.127
	152	0.047	0.000	0.035
EBmac0806	167	0.065	0.143	0.085
	165	0.000	0.371	0.092
	161	0.056	0.000	0.042
	159	0.121	0.229	0.148
	158	0.757	0.114	0.599
	156	0.000	0.143	0.035
HVHVA1	121	1.000	0.886	0.972
	115	0.000	0.114	0.028
HvLOX	152	0.000	0.057	0.014
	150	1.000	0.943	0.986
Bmag0223	183	0.000	0.057	0.014
	176	0.000	0.057	0.014
	174	0.000	0.314	0.077
	169	0.056	0.114	0.070
	167	0.318	0.000	0.239
	165	0.178	0.000	0.134
	163	0.037	0.229	0.085
	161	0.084	0.000	0.063
	159	0.131	0.029	0.106
	158	0.075	0.057	0.070
	157	0.028	0.029	0.028
	155	0.000	0.114	0.028
	153	0.093	0.000	0.070
HVM62	275	0.187	0.000	0.141
	273	0.486	0.000	0.366
	267	0.318	0.057	0.254
	265	0.000	0.114	0.028
	262	0.000	0.029	0.007
	258	0.009	0.714	0.183
	241	0.000	0.086	0.021
HVM54	162	0.037	0.086	0.049
	160	0.093	0.000	0.070
	159	0.000	0.886	0.218
	154	0.505	0.000	0.380
	151	0.364	0.000	0.275

Appendix-V Cont.

SSRs	Alleles (bp)	Frequency		
		NL	GC	NL+GC
HVM54	111	0.000	0.029	0.007
Bmag0007	225	0.000	0.057	0.014
	218	0.000	0.086	0.021
	214	0.009	0.029	0.014
	213	0.028	0.000	0.021
	208	0.009	0.000	0.007
	205	0.009	0.000	0.007
	203	0.028	0.000	0.021
	202	0.009	0.000	0.007
	201	0.178	0.000	0.134
	198	0.159	0.200	0.169
	196	0.290	0.000	0.218
	194	0.103	0.571	0.218
	192	0.028	0.000	0.021
	190	0.056	0.057	0.056
	188	0.009	0.000	0.007
	184	0.065	0.000	0.049
	183	0.019	0.000	0.014
Bmac0032	252	0.009	0.000	0.007
	251	0.019	0.000	0.014
	248	0.009	0.000	0.007
	246	0.009	0.000	0.007
	243	0.093	0.171	0.113
	241	0.075	0.000	0.056
	240	0.084	0.000	0.063
	239	0.075	0.000	0.056
	237	0.093	0.000	0.070
	234	0.075	0.000	0.056
	231	0.009	0.000	0.007
	220	0.037	0.143	0.063
	218	0.009	0.057	0.021
	216	0.000	0.314	0.077
	214	0.000	0.286	0.070
	213	0.000	0.029	0.007
	212	0.065	0.000	0.049
	209	0.336	0.000	0.254
Bmac0040	239	0.000	0.029	0.007

Appendix-V Cont.

SSRs	Alleles (bp)	Frequency		
		NL	GC	NL+GC
Bmac0040	235	0.000	0.029	0.007
	230	0.000	0.343	0.085
	228	0.000	0.029	0.007
	227	0.000	0.057	0.014
	224	0.000	0.057	0.014
	207	0.000	0.171	0.042
	204	0.000	0.029	0.007
	179	0.991	0.029	0.754
	177	0.009	0.229	0.063

Appendix-VI UPGMA clustering groups of the 161 barley genotypes

Group	Genotypes	Group	Genotypes	Group	Genotypes
(Ia)	Alexis		Thonje-3		Pork-1
	Freedom		Thangja-1		Pork-2
	McGwire		Bimtakothi-1		Tsumje-2
	Candle		Bimtakothi-5		Pisang-5
	Alamo		Bimtakothi-2		Pisang-8
	Silky		Bimtakothi-4		Pisang-6
			Bimtakothi-12		Pisang-7
(Ib)	Ludmilla		Thonje-6		Pisang-9
	Verena		Thonje-5		Ghara-1
	Anson Barley		Bimtakothi-10		Ghara-2
	Russia 32		Bimtakothi-3		Sikha-1
	Bulgarian 347		Bimtakothi-11		Sikha-5
	Krasnodar 1920		Bimtakothi-9		Sikha-2
	Carola		Thangja-3		Sikha-6
	Andrea		Thonje-4		Sikha-7
	Theresa		Bimtakothi-13		Solu Uwa
	Cita		Chame-3		Nepal-1
	Jana		Thonje-16		Nepal-2
	Tapir		Tilman Camp-7		Sikha-8
	Dura		Chame-11		Sikha-4
	Franka		Chame-13		Ulleri-9
	Birgit		Chame-12		Ulleri-21
	Vogel Agaer		Thangja-2		N-6
	Friedrichswerther Berg		Tilman Camp-8		N-12
	Peragis Mittelfrüh		Chame-2		Nepal-3
	Mausberg		Pisang-4		Nepal-4
	Derenburger		Lih D. Gal		Nepal-5
	Mahndorfer		Sama-8		Nepal-6
	Hauters Wintergerst		Ngyak-11		Nepal-7
	Tschermaks		Sama-3		Sipche-2
	Gerbel		Sama-9		Sipche-3
	Vogelsanger Gold		Chame-8		Sipche-4
	Mädru		Chame-9		Sipche-6
	Mammuth		Dhumpu-2		Sipche-7
	Tokyo		Jomsom-1		Sipche-9
	Tessi		Jomsom-2		Sipche-11
	Danilo		Kagbeni-3		Philem-1
	Malta 1970er		Kagbeni-5		Philem-2
	Malta		Tukucha		Philem-3
	Sonja		Phalatey		Sama-1
	Hanna		Thonje-21		Sama-2
	Igri		Tsumje-1		Sama-6
	Marinka		Ngyak-10		Thomje-2
	Opal		Gho-1		Thomje-3
	Angora		Gho-2		Sipche-12
	Misato Golden		Ngyak-4		Thomje-4
	Rest. Ym No.1		Ngyak-3		Thomje-7
			Ngyak-1		Sama-4
(Ic)	09-01		Chame-14		
	09-09		Thonje-19	(IIb)	Mokusekko 3
			Thonje-18		Taihoku A
(IIa)	Annapurna BC-1		Naked-304		Chikurin Ibaraki 1
	Annapurna BC-2		Thomje-6		Ea 52
	Tilman Camp-1		Gho-3		Muju covered 2
			Ngyak-12		Russia 57
			Ngyak-2		

Genotypes are in original order of the dendrogram (Fig. 13) from the top (left) to the bottom (right)

Curriculum vitae

Name:	Madhav Prasad Pandey
Date of Birth:	05 November 1970
Place of Birth:	Birgha-6 Marengdi, Syangja, Nepal
Nationality:	Nepalese
Marital Status:	Married
Dependents:	two
Address:	Home: Birgha Archale VDC-6, Marengdi, Syangja, Nepal Office: Department of Plant Breeding Institute of Agriculture and Animal Science (IAAS) Rampur, Chitwan, Nepal
Education	School Leaving Certificate (SLC) 1986 Ministry of Education, Govt. of Nepal I Sc Agriculture 1988 Tribhuvan University, Institute of Agriculture & Animal Science, Rampur, Nepal B Sc Agriculture 1992 Tribhuvan University, Institute of Agriculture & Animal Science, Rampur, Nepal M Sc Agriculture (Plant Breeding) 1997 Rajendra Agricultural University, Samastipur, India
Employment	Technical Officer: Nepal Agricultural Research Council (1993-94) Research Associate cum Collaborator: Directorate of Research, Institute of Agriculture & Animal Science (1997-99) Lecturer of Plant Breeding: Department of Plant Breeding, Institute of Agriculture & Animal Science (1997 to date)
Trainings	Advanced professional training in biotechnology DSE/ZEL, Germany (August to December, 1999) Molecular Bioinformatics IFZ Graduate training course (May 30 to June 3, 2005)

Declaration

I hereby declare that the thesis entitled “Molecular assessment of genetic diversity and population differentiation of hulless barley (*Hordeum vulgare* L.) landraces from the Himalayas of Nepal, and its relevance for barley breeding” is my original work, except otherwise acknowledged in the text. I have not submitted this thesis or part of it for credit towards a degree to any other institution.

Madhav Prasad Pandey

Giessen, 01 December 2006

ISBN: 978-3-86727-112-7