Barley Genetic Resources for Kyrgyz Plant Breeding

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Cover: Barley cultivation fields in Kyrgyzstan, barley spikes, seed collecting and field trials

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Abstract

Barley (*Hordeum vulgare* L.) is one of the most important forage cereal crops in Kyrgyzstan. Today it is grown under extremely hard conditions in many highland regions where other crops cannot compete. Kyrgyzstan is a mountain agrarian country and agriculture is one of the major sectors of the economy. Almost 65% of the Kyrgyz population is living in rural area. Currently, many local farmers are facing various problems in order to find suitable cultivars which can grow under the harsh conditions with capacity of high yield and good resistance to abiotic and biotic stresses. In most cases modern barley cultivars are limited ability to grow and to have the potential of a high yield under such hard environment conditions. Many farmers grow unknown material of barley, which has some useful agronomic traits that could give a good yield under a short vegetation period in highland areas.

The aim of this doctoral thesis was to evaluate the phenotypic and genotypic diversity of spring barley (*Hordeum vulgare* L.) grown in Kyrgyzstan and other materials estimating their potential usefulness for breeding in Kyrgyzstan. In the present study morphological and microsatellite markers as well as agronomic traits were used in order to characterize the diversity of the studied accessions. The results facilitate a better understanding of the genetic diversity level and relationships of barley material from different eco-geographic regions. High genetic diversity was found among the collected 'farmers' mixture populations' (FMP) used by Kyrgyz farmers as compare with the rest of studied materials. Cluster analysis shows a clear separation between farmers' material and other bred cultivars and landraces. Though for the majority of FMPs material origin could not be identified, some individuals showed to be closely related with Kyrgyz bred cultivars. When agronomic performance of subset of accessions was evaluated there were some cultivars and landraces identified which showed more stable agronomic characteristics in different agro-environment conditions. These cultivars can be used in Kyrgyz barley breeding program as sources of traits for cultivar improvement.

Keywords: Genetic diversity, FMPs, breeding, molecular marker, SSR, Kyrgyzstan

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Dedication

This thesis dedicated to my parents: Kubat and Zhenish, to my own family with gratitude for your unfailing love, passion and great support, and for believing in me throughout my study period.

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Usubaliev B., Brantestam A., Salomon B., Garkava-Gustavsson L., Bothmer R. (2013). Genetic diversity in farmer grown spring barley material from Kyrgyzstan. *Genetic Resources and Crop Evolution*: 1-16. doi:10.1007/s10722-013-9959-2

II Usubaliev B., Brantestam A., Salomon B., Garkava-Gustavsson L., Bothmer R. A comparison of genetic diversity in bred cultivars and currently growing farmers' mixture population (FMPs) of spring barley from Kyrgyzstan (*Submitted*)

III Usubaliev B., Brantestam A., Salomon B., Garkava-Gustavsson L., Bothmer R. Genetic relationship among the spring barley cultivars from Kyrgyzstan and North European and West-central Asian barley as indicated by microsatellites (*Manuscript*)

IV Birzhan Usubaliev., Agnese Kolodinska Brantestam., Björn Salomon., Skaidrite Bumane., Larisa Garkava-Gustavsson., Roland von Bothmer. Agronomic performance of spring barley cultivars under different eco-environment condition (*Manuscript*)

The contribution of Birzhan Usubaliev to the papers included in this thesis was as follows:

I. Planned the study, collected the accessions and planted seedlings in a greenhouse, extracted DNA and all laboratory work carried out. Made the data analyses and wrote the manuscript in cooperation with the co-authors

II. Planned the study, planted seedlings in a greenhouse and made DNA extraction from collected young leaf tissues. Carried out all laboratory work, analysed the data and wrote the manuscript in cooperation with the co-authors

III. Planned the study, set up the experiments, carried out all field experimental work, evaluated and analyzed the data and wrote the manuscript with input from the co-authors

IV. Planned the study, assessed and analyzed the data and wrote the manuscript in cooperation with the co-authors

Abbreviations

PCR	Polymerase chain reaction
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphates
CTAB	cetyltrimethylammonium bromide
SSR	Simple sequence repeat
UPGMA	Unweighted pair group method with arithmetic mean
NTSYS	Numerical Taxonomy System
FMP	Farmer' Mixture Population
PCA	Principal component analysis
PCoA	Principal coordinate analysis
SCVT	State Commission of Variety Testing

1 Introduction

Barley (*Hordeum vulgare* L.) is one of the most ancient crops among the cereals and has played a significant role in the development of agriculture (Ullrich, 2011). Today, cultivated barley is grown in much more diverse eco-geographic environmental conditions as compared to other crop species. It can be planted from the tropics to marginal areas in North and high altitudes (Nevo & Shewry, 1992). Before the 20th century barley was mainly used as a human food but presently it is used mainly as animal feed. It is also used for malt production and human consumption. Barley, in comparison with other cereal crops has a better fodder value including both grain and straw. In most developed countries barley straw is used for animal bedding, whereas in the developing counties it is also used for animal feed (Akar *et al.*, 2012).

1.1 Taxonomy

Barley belongs to the genus *Hordeum*, which is a moderately sized genus with ca. 32 species and altogether ca. 45 taxa (Bothmer *et al.*, 2003c). All species have the basic chromosome number of x=7. Cultivated barley, *Hordeum vulgare* ssp. *vulgare*, and its wild ancestor *H. vulgare* ssp. *spontaneum* (K. Koch.) Asch. & Graebn. are diploid taxa with 2n=2x=14 chromosomes. Other *Hordeum* species are diploid, tetraploid (2n=4x=28) or hexaploid (2n=6x=42) (Komatsuda *et al.*, 1999). Morphologically the *Hordeum* species are rather similar. Plants usually have three, one-flowered spikelets at each rachis node, called triplets. The two lateral florets are pedunculate, or sessile, and may be sterile as in two-rowed barley or fertile as in six-rowed barley (Bothmer *et al.*, 2003a). According to Harlan's gene pool concept all barley species can be

divided into three main genepools defined for barley by Bothmer *et al.* (2003a) (Figure.1). The primary genepool includes elite breeding materials, cultivars, landraces and the wild ancestor of cultivated barley, *H. vulgare* ssp. *spontaneum*. There is no barrier for gene transfer within the primary genepool. The secondary gene pool includes only one species, *H. bulbosum* L., which shares the basic **H** genome with barley. The crossing ability between these two species is difficult but gene transfer is possible. Commonly, the '*bulbosum*' chromosomes are eliminated after pollination of *H. vulgare* with *H. bulbosum* and this phenomenon is used for production of doubled haploids in barley breeding. The tertiary gene pool of barley is very large and includes all other remaining wild *Hordeum* species and these have a strong crossability barrier with cultivated barley (Bothmer *et al.*, 2003a). However, the gene transfer between distantly related species can be conducted by means of biotechnology methods (Ceccarelli *et al.*, 2008; Bothmer *et al.*, 2003a)

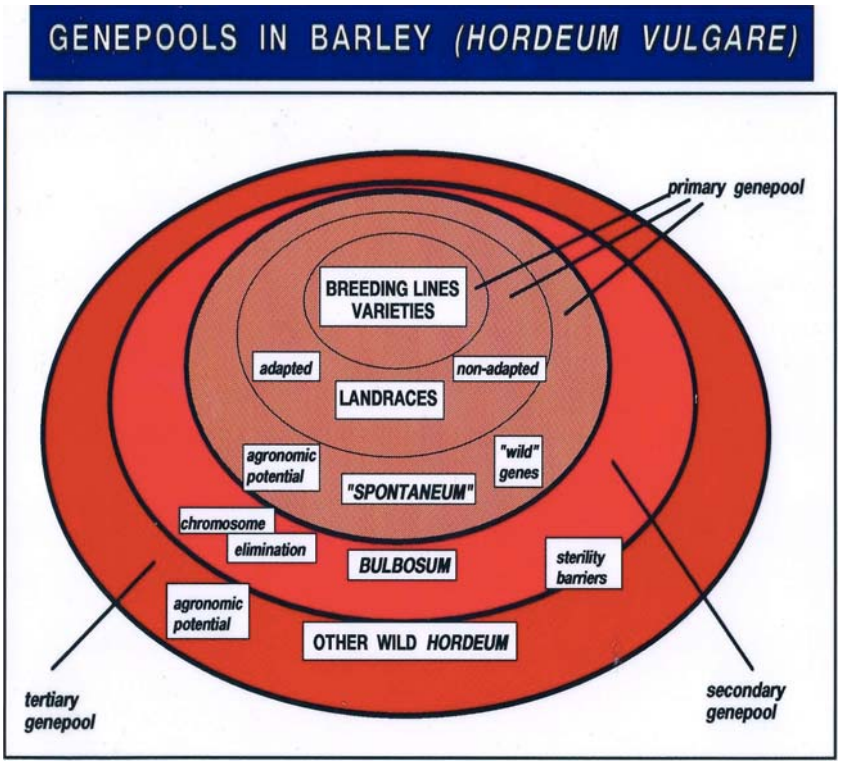

Figure 1. Genepools in cultivated barley (*Hordeum vulgare*) (Bothmer *et al.*, 2003a)

1.2 Center of origin and domestication

The center of origin of crops may be defined as regions where a crop was initially domesticated and where the wild progenitor and the derived cultivated species exist (Molina-Cano *et al.*, 2005). The information about domestication of barley has a fundamental importance for understanding of the origin and early distribution of agrarian culture (Morrell & Clegg, 2007). The single progenitor of the cultivated barley is *Hordeum vulgare* ssp. *spontaneum*, which is still abundant in nature. It was discovered and described by Karl Koch from materials collected in Turkey. Cultivated barley (*Hordeum vulgare* L. ssp. *vulgare*) was domesticated around 10,000 years ago. There is clear evidence that barley originated from the Eastern Mediterranean in an area called the Fertile Crescent, which is covering geographic areas in Israel, Jordan, Lebanon, Syria, southeastern Turkey, Northern Iraq, western Iran (Azhaguvel & Komatsuda, 2007; Blattner & Badani Mendez, 2001; Badr *et al.*, 2000; Zohary & Hopf, 1988). However, recently Orabi *et al.* (2007) claimed that there might be another independent domestication site of barley in Eritrea and Ethiopia and they consider that this geographical region is at least a center of diversification of barley.

Figure 2. The Fertile Crescent, the area early for domestication of cultivated barley (*Hordeum vulgare ssp, vulgare*) (Feuillet *et al.*, 2008)

1.3 Barley cultivation worldwide

Cultivated barley (*Hordeum vulgare* L.) is one of the first domesticated crops having long history of adaptation to cultivation worldwide (Bothmer *et al.*, 2003b). Barley is grown in a wide eco-geographic range around the world and is thus one of the best-adapted crops to diverse cultivation conditions. Barley fields can be found from the tropics to high latitudes (>60°N) in Iceland and Scandinavia as well as in high altitudes up to 4,500 m.a.s.l in the Himalayas (Ceccarelli *et al.*, 2008; Bothmer *et al.*, 2003b; Nevo & Shewry, 1992). In developing countries barley cultivation dominates in arid and semi-arid climates where it sometimes is the only staple food resource.

It has a comparably stable yield in spite of climatic variation within the growing season. In this respect wheat and other small grain cereals cannot compete with barley (Goyal & Ahmed, 2012; Stefansson *et al.*, 2012). On a global scale barley is known to be a drought, cold and salt tolerant crop and adapted to low-input environmental conditions. And as mentioned above barley can grow at extremely high altitudes. For these reasons barley is a very important feed crop in mountainous countries in Asia, Africa and South America (Fischbeck, 2002). In most developing countries barley can reduce the risk of a very low yield or crop failure for poor farmers (Akar *et al.*, 2012; Newton *et al.*, 2011). For example, in South America, Central Asia, Middle East, Tibet, Nepal and North Africa barley is grown at high altitudes and commonly under rain-fed conditions (Upreti, 2005).

Barley can produce a high yield under ideal cultivation, such as moderate rainfall (400-800mm), well-drained loamy soil, irrigation, and moderate temperature regimes (15-30^{0}C) (Ullrich, 2011). However, barley is regarded as one of the most tolerant crops to drought and alkaline soils and it has the highest water-use efficiency compared to other cereal crops but it is less tolerant to acid soils (Newton *et al.*, 2011; Cossani *et al.*, 2009; Poehlman, 1985). .

Barley has a considerable economic importance in agriculture and industry in many countries. The total barley grain production around the world accounted for 134 million tonnes in 2011 (FAOSTAT, 2011). According to the United Nations Food and Agriculture Organization's (FAO) database barley in recent years has became the fifth most important produced crop in the world after wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) rice (*Oryza sativa* L.) and soybeans (*Glycine max* (L.) Merr.) (FAOSTAT, 2011). The largest barley producers in the world are France, Germany, Russia, Spain, Ukraine, Canada, Australia and Turkey. In total 134.3 million tonnes of barley grain (Figure 3) was produced in 2011, which corresponds to a cultivation area of about 48.6

million hectares. The barley production is not evenly distributed over the world. In 2011 the European countries produced about 81.3 million tonnes. The second largest production was in Asian countries with about 21.2 million tonnes. The third area in barley production was North and South America with 16.6 million tonnes. In Oceania about 8.3 million tones and in Africa about 6.8 million tonnes were produced. The total production of barley grain in Central Asia was only 3.1 million tonnes.

The largest barley grain exporters were European countries e.g., France exported 6.4 million metric tonnes. There are other leading barley exporters like Ukraine (3.6 M /t), Germany (1.3 M/t), Canada (1.5 M t), Russia (3.4 M/t), and Australia (3.2 M/t). All these export countries have special focus on malting barley. In total 29.6 million metric tonnes of barley grain was exported in 2011 in the world. The FAO statistic data showed that the leading importer countries are from Asia and Africa. The major barley importer is Saudi Arabia with 7.0 million metric tonnes in 2012. Other countries like China (1.8 M/t), Japan (1.3 M/t), Iran (1.2 M/t) and Morocco (2.7 M/t) are considered as top importers.

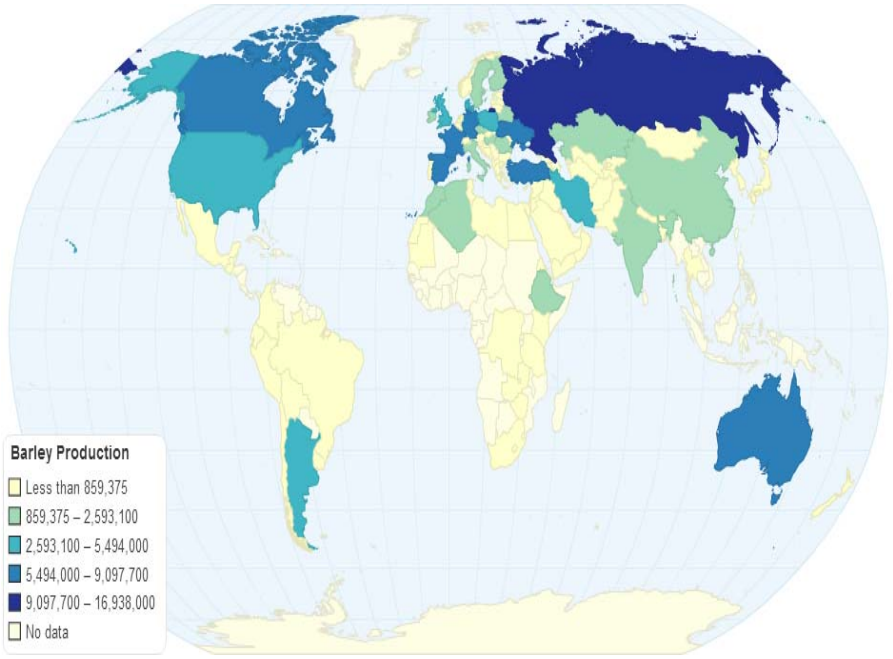

Figure 3. Map of barley production in the world (207-2011). Source: http://faostat.fao.org

1.4 Genetic diversity in barley

Genetic biodiversity and its utilization are topics for a wide range of research (Bothmer *et al.*, 2003b), since crop genetic diversity of crops is considered to be one of the main resources supporting human life. Genetic diversity of any crop species is defined as genetic variation within and between populations, landraces and cultivars, arising due to recombinations, mutations, and introgressions (Hawkes, 1983). The use of highly diverse germplasm in breeding increases the chances for success in developing highly productive new cultivars with good quality properties over a long period of time (Bockelman *et al.*, 2010; Horsley *et al.*, 1995). Genetic diversity of related wild species or crop ancestors can also be important to use in breeding in order to solve problems related to crop failure (Geleta, 2007). Genetic diversity in barley is preserved in genebanks (*ex-situ*) and in nature (*in-situ*). A total number of more than 400 000 barley accessions are available for research and breeding at different genebanks in the world. Gene bank collections represent landraces (44 %), breeding lines (17 %), crop wild relatives (15 %), cultivars (15 %) and genetic stocks (9 %) (Bockelman *et al.*, 2010). The largest collection (ca. 40 000 accessions) of barley is held in Canada (Plant Gene Resources of Canada). The second largest collection is in United States (ca. 30 000 accessions) (USDA-ARS National Small Grain Collection), whereas the third biggest collection (ca. 30 000 accessions) is held in Brazil (Recursos Geneticos e Biotecnologia, EMBRAPA/CENARGEN), followed by collection in ICARDA (Syria) with ca 26 000 accessions (Bockelman *et al.*, 2010)

1.5 Barley in Kyrgyzstan

1.5.1 Production and use

In Kyrgyzstan during the last five years barley was cultivated on around 125.4 thousand hectares (Table 1), and the total production was 238.6 thousand tonnes (FAOSTAT 2011). Nowadays, barley is here grown under extreme eco-environmental conditions (Usubaliev *et al.*), since more favorable areas are occupied by wheat cultivation which is considered to be a strategic crop for food security in the country.

Barley is the main feed crop in Kyrgyzstan, though it is also used for human consumption (Usubaliev *et al.*, 2013). Barley grain was used as food by the ancient nomad Kyrgyz people in the form of non-alcohol thirst quenching

drinks by historic names (Zharma, Maksym, Achytma) which nowadays is becoming increasingly popular in the Kyrgyz market (Usubaliev *et al.*, 2013). In the animal husbandry sector barely grain is the most important feed source for livestock including dairy cattle, sheep, beef, pigs and poultry. However, barley straw is also used as feed and in many cases used mixed either with silage or with dry hay (Bessonova, 2007).
Annual production during the last five years has been an average of 238.6 thousand tonnes per year, of these 85 % is used as feed for animal, 9 % for beer production and remaining 6 % is as food (FAOSTAT, 2011) (Table 1).

Table 1. Barley production in Kyrgyzstan (2007-2011)

Year/Crop	2007	2008	2009	2010	2011	Average
Area Harvested (K ha)	125.4	133.3	123.6	122.5	122.0	125.4
Production (K t)	227.2	210.6	289.7	231.5	233.8	238.6
Yield (t/ha)	1.8	1.6	2.3	1.9	1.9	19.1
Beer of barley (K/t)	14.0	15.4	15.1	18.0	21.1	14.0
Food supply (K/t)	4.00	6.2	5.6	-	-	-

K, thousands; t, metric tonnes. Source: FAO

1.5.2 Breeding

Barley played an important role in the beginning of 20th century in Kyrgyzstan when the only cereals grown here were barley and millet. In total 558.6 thousand hectares were planted with cereal crops with an average yield of 0.8 t/ha in 1913 (Tursunov, 1977). The first provincial experimental trial station was established in 1926 and later reorganized as the Kyrgyz state breeding station in 1927. The first barley collection expedition for local material was organized in 1934. Lyashenko (1935) reported that during this expedition around twenty local landraces of barley from different eco-geographic zones of the country were collected. Among the collected barley landraces the most common ones were known by the names ‘Ak-Arpa’ (white barley), ‘Sary-

Arpa' (yellow barley), 'Shaly-Arpa' (Shaly barley), and 'Kyrgyz-Arpa' (Kyrgyz barley). Most of these local landraces represented mixtures of different botanical types, e.g. mixtures of two- and six-rowed barley. The first bred cultivar 'Persicum 64' from Krasnodar (Russia) was released for cultivation on territory of Kyrgyz Republic in 1934 and the first period of breeding activities finished (Majstrenko, 1954). The second stage of breeding activities started in 1938 and involved material from the Research Institute of Plant Industry (nowadays N.I.Vavilov Research Institute of Plant Industry in Russia) as well as local material. Between 1944-1955 four barley cultivars resulting from the national breeding program were transferred to the commission of variety testing ('Nutans 187', 'Pallidum 1507', 'Nutans 1071', 'Nutans 45' and 'Kyrgyzskij 45'). Among them the cultivar 'Nutans 187' stayed for a long period in cultivation (Majstrenko, 1954). The next step in Kyrgyz breeding was initiated in the mid 1950's when breeders started to use intraspecific crosses and already at that time around 120-150 thousand hectares was grown with new barley cultivar as a result of national breeding and as well as breeding in other Soviet Republics. During the existence of the Institute of Farming 29 cultivars of spring and winter barley were released, which are still the most grown cultivars in Kyrgyzstan. In the end of the 20th century international collaboration was started, for example with ICARDA. Additional types of material from ICARDA were included in the national breeding program (Akimaliev, 2006). In 2011, 23 (8 of these are Kyrgyz cultivars) spring barley cultivars and 7 (5 of these are Kyrgyz cultivars) winter barley cultivars were grown in the country. These are listed in State Commission of Variety Testing (SCVT, 2010).

2 Objectives of the study

The primary aim of this doctoral thesis was to evaluate the phenotypic and genotypic diversity of spring barley (*Hordeum vulgare* L.) grown in Kyrgyzstan and other martials estimating their potential usefulness for breeding in Kyrgyzstan. Specific objectives were the following:

1. To characterize the genetic diversity of spring barley currently grown in Kyrgyzstan

2. To estimate the genetic diversity and relationships of bred cultivars and farmers' mixture populations from Kyrgyzstan

3. To analyze the agronomic performance of spring barley material under different agro-environmental conditions

4. To compare the genetic diversity of spring barley materials from Kyrgyzstan with materials from some European and Asian countries.

3 Materials and methods

3.1 Plant material

The Barley germplasm used in this study, included farmers' grown material from Kyrgyzstan, landraces from Russia and Central-West Asia, as well as advanced bred barley cultivars from Kyrgyzstan, Ukraine, Nordic and Baltic countries.
The Farmers' Mixture Populations (FMP) were collected from different farmers' fields representing two mountain provinces (Issik-Kul, and Naryn at 1600 and 2300 m.a.s.l), whereas landraces and cultivars of different country of origin were obtained from the Nordic Genetic Resource Center (www.nordgen.org), N.I Vavilov Institute of Plant Industry (http://vir.nw.ru) and from Kyrgyz Research Institute of Farming.

Twenty-two FMPs of spring barley was used for the genetic diversity study (Paper I).
Twenty-one Farmers' Mixture Populations (FMP) of spring barley and nine improved cultivars from Kyrgyzstan and one from Ukraine (which is cultivated in Kyrgyzstan) were used in Paper II.

Paper III included eighteen, two-rowed and two six-rowed bred cultivars and nine landraces of spring barley.

Sixty-one spring barley accessions including twenty-one Farmers Mixture Populations (FMP) and another forty accessions representing different countries were used in Paper IV

3.2 DNA extraction

Barley seedlings were planted in a greenhouse and the second fresh leaf was harvested and placed in eppendorf tubes, frozen in liquid nitrogen and freeze-dried for 48 hours. All dry samples were crushed using the Retsch shake equipment for 4 minutes at 15 rpm. DNA was extracted by a modified CTAB-method (Cheng *et al.*, 2003). After extraction the quality of DNA was estimated using NanoDrop ND-1000 spectrophotometer and by electrophoresis on 1.7 % agarose gel.

3.3 SSR analysis

Polymerase chain reaction (PCR) was performed by means of 13 selected fluorescent-labelled SSR (microsatellite) primer pairs (Bmac0032, Bmac0273, EBmac0701, EBmac0040, Bmag0013, Bmag0007, Bmag0173, Bmac0067, Bmag0135, EBmac0970, Bmag0384, Bmac0399, AF43094, HVM36). The SSR loci studied are located on different chromosomes and chromosome arms. The PCR reaction was carried out in 10 µL volume containing PCR buffer (Applied Biosystem), 0.2 nM d'NTP, 0.25 µM of each forward and reverse fluorescent-labelled primer and 2.0 mM $MgCL_2$, 0.001 U Tag and 40 ng DNA. The PCR programme was carried out according to Ramsay *et al.* (2000). Electrophoresis of PCR products was conducted at the Clinical Chemistry DNA laboratory (Malmö, Sweden) using an Applied Biosystems 3130 Genetic Analyser. The allele size was analysed using the Peak Scanner software v.1.0.

3.4 Field experiments and agronomic traits

The field trials were carried out in two different agro-environmental climatic zones: one is in Priekuli, Latvia (57°19'N, 24°20'E, altitude 20 m.a.s.l) and a second in Issyk-Kul, Kyrgyzstan (42° 3'59.49'N, 76°53'9.59'E, altitude 2000 m.a.s.l). The experiment layout was a Randomized Complete Block Design using two replicates and two years in both countries. The phenological observations of agronomic traits and for biometrical measurements were carried out according to international classification system for *Hordeum* L. (Trofimovskaya, 1974). The five agronomic traits, heading day (HD), plant height (PH), spike length (SL), number of gains in spike (NGS) and thousand kernel weight (TKW) were evaluated either in laboratory or directly in the field

conditions. The details about these agronomic characteristics and the experiment field layout are described in paper III.

3.5 Data analysis

The diversity of each locus was described by the genetic diversity index (h) as: $h=1-\sum Pi^2$, where Pi is the frequency of the *i*th allele of the locus (Nei, 1978). Total genetic diversity for the groups of accessions (H) was calculated from polymorphic loci according to Hamrick and Godt (1997). Genetic diversity index calculations and determination of number of alleles were made using Popgene software (Yeh, 1997). The NTSYSps software (Rohlf, 2000) was used to calculate genetic distance based on the Rogers-Wright coefficient (Wright, 1978) and to perform principal co-ordinate analysis (PCoA). Analysis of molecular variance (AMOVA) using Arlequin software version 3.5.1.2 (Schneider *et al.*, 2000). The Minitab 16.0 software package program (Ryan, 1986) was used for statistical Analysis of Variance. For the comparison of agronomic traits between years, a paired t-test was used for each cultivar whereas, between countries for each cultivar was applied General Linear Model.

4 Results and Discussions

4.1 Phenotypic diversity (paper I)

The Farmers' Mixture Populations (FMP) of Kyrgyzstan were found to be very diverse. The diversity of FMPs could be explored as a potential source of traits for crop improvement. The within accession diversity should be considered when developing *ex-situ* conservation and also when using germplasm in breeding (Paper I). High variation of several morphologic traits was observed like e.g. spike type (two and six-rowed), color of spike (yellow, dark yellow, brown, and violet), and spike length (short, long) (Figure 4). In the studied material the majority of FMPs (78 %) represented two-rowed barley whereas only 22% was six-rowed barley. The predominant spike color of the FMPs' was yellow (72 %). The most common spike length (70 %) varied from five to nine cm. About 46 % of two-rowed accessions had from 17 to 20 kernels per spikes. Though overall Kyrgyz farmers' material showed a great diversity in morphological traits, some accessions were morphologically more uniform in the fields and resembled advanced cultivars.

Figure 4 different morphological spike types of FMPs

4.2 Molecular genetic diversity (paper I-II-IV)

Before the DNA marker technologies were available, the conventional methods of germplasm characterization were used in order to classify barley materials for breeding e.g. morphological traits, pedigree information and some biochemical analyses. However, the traits used by conventional methods are often influenced by environmental factors and depend on development stages of the plants (Pham *et al.*, 2009; Matus & Hayes, 2002). A number of molecular markers including restriction fragment length polymorphism (RFLP), random amplified fragment polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) have been widely used to estimate the genetic diversity of crop resources (Tanto Hadado *et al.*, 2009; Leisova *et al.*, 2007; Varshney *et al.*, 2007; Russell *et al.*, 2003; Czembor *et al.*, 2002; Liu *et al.*, 1996). To characterize the genetic diversity of Farmers' Mixture Populations (FMP) of spring barley from Kyrgyzstan the SSR marker system was used. These markers have advantages owing to its co-dominant inheritance and highly variable repeats in the genome (Zhang *et al.*, 2005; Gupta *et al.*, 2003; Johansson *et al.*, 1999). Fourteen SSR loci were studied and revealed a different degree of polymorphism and number of alleles per locus (Paper I). No heterozygous individuals were found in the studied material based on molecular markers. To use more than one method for genetic diversity analysis of crop species allows better description of the studied material and better understanding of the genetic variation (Pham, 2011). We combined the morphological and molecular markers and according to a PCoA study based on SSR data, there was less distinct separation between two- and six-rowed types where other researchers working with material of different origins showed a very clear separation among six- and two-rowed barleys based on DNA markers (Chen *et al.*, 2010; Ordon *et al.*, 2005). Landraces and commercial cultivars showed a clear distinction between two- and six-rowed barley (Lasa & Igartua, 2001). Chaabane *et al.* (2009) using SSR markers evaluated landraces from Tunisia and found two clearly distinct groups according to row type. In the current study the genetic diversity between morphological and molecular markers showed that the lower genetic diversity displayed by SSR markers in material corresponds to phenotypically more uniform materials. Several studies of genetic diversity in different crops have shown a high correlation between genetic and morphological markers (Pejic *et al.*, 1998; Russell *et al.*, 1997; Kantety *et al.*, 1995). However, in our study only four morphological traits were used that can limit the possibility to discriminate the material. Nevertheless, high genetic diversity was found within accessions but variation it is not large between accessions. This explains

that farmers' material from different areas is genetically similar and comprises several strains.

The genetic diversity estimation indicated that FMPs had higher genetic diversity (H=0.653 A=8.5) than bred cultivars (H=0.573, A=3.8) (Paper II). The highest diversity was observed in the loci Bmag0007 (h=0.846, A=15) and Bmag0399 (h=0.821, A=15), respectively. The genetic variation within the accessions showed higher values in average in FMPs (Hs=0.385, A=2.6) whereas, the average value of bred cultivars was only (Hs=0.066, A=1.1). The overall average genetic diversity (H) in our study was 0.678 with an average of 9.0 alleles per locus. Corresponding results were obtained by Russell *et al.* (1997) analysing European barley cultivars and landraces with an average genetic diversity of H=0.57 and in average 7.5 alleles per locus. Backes *et al.* (2009) reported that genetic diversity of Eritrean farmers' material had in average H=0.51 with 4.4 alleles. However, the higher genetic diversity of FMPs in Kyrgyzstan indicates that they include different strains of barley, which are morphologically and genetically different whereas bred cultivars showed more uniformity within the accessions as expected.

In Paper IV, 12 SSR loci in 486 individuals from 61 barley accessions from different countries (Kyrgyzstan (30), Sweden (5), Latvia (1), Norway (1), Russia (10), Estonia (1), Afghanistan (2), Pakistan (1), Ukraine (1) and China (7) revealed a total number of 110 alleles. The number of alleles per locus varied from 3 (Ebmac0970) to 19 (Bmac0032) with an average of 11.8 alleles per locus. Other studies have shown that the total number of alleles depend on type (two- or six-rowed) and on geographic origin of the used material (Wang *et al.*, 2010; Struss & Plieske, 1998). The genetic diversity value (H) differed between groups, where the highest genetic diversity value within accessions (Hs=0.425) was obtained in FMPs accessions collected from the farmers' field in Kyrgyzstan. The landraces from Russia (Hs=0.131) and accessions from Afghanistan, China and Pakistan (Hs=0.116), showed lower within accession diversity. The lowest within accession diversity was found in cultivars from Kyrgyzstan (Hs=0.066) and Nordic and Baltic countries (Hs=0.034).

4.3 Genetic relationship of barley accession from different eco-geographic regions (paper I-II-IV)

The genetic diversity of barley accessions representing different eco-geographic groups was discussed in paper I, II and IV. In our study the PCoA

analysis of FMPs there was not a clear grouping related to their geographic origin and the separation between two- and six-rowed types did not show a clear separation as reported in other studies (Chaabane *et al.*, 2009). A possible reason for the FMPs not to be differentiated based on their geographic origin is that active exchange and mixture materials among farmers across region may have occurred (paper I). Based on UPGMA cluster analysis on accessions level the genetic distances between FMPs and bred cultivars from Kyrgyzstan the dendrogram was divided into six clusters at genetic distances 0.72 and in 18 sub-clusters and distance more than 0.62 The FMPs were separated in a sub-cluster and landraces also were grouping in number of sub-clusters depending on their geographic origin. Among the bred cultivars clustering demonstrated that accessions group according to their agronomic characteristics like, e.g. cultivars used in rain fed vrs. irrigated land and there was one sub-cluster of cultivars that are recommended for high mountain provinces.

4.4 Agronomic evaluation of barley cultivars in different eco-geographic conditions

According to PCA analysis based on all five agronomic characteristics the first component distinguished two-and six-rowed types. In this study the separation between two-and six-rowed cultivars and landraces were affected mainly by number of grains in spike (NGS) and heading date (HD) whereas, the second component was affected by the spike length (SL), HD and thousand kernel weight (TKW).

The ANOVA analysis of all five agronomic parameters in the studied material showed a significant difference between cultivars and landraces. There was a significant effect of the environment x genotype interaction for all traits of studied material and also interaction of genotype x year (location) was detected. However there was no significant difference between different years within the same year trail locations for the NGS trait. Corresponding results were reported by Dofing *et al.* (1992) studying environment interaction in advanced bred cultivars of spring barley. They found and there was a significant interactions for environment x genotype in different climatic conditions. In our study the major environment effect between Latvian and Kyrgyzstan is the distinct differences in day-length. For example, in Latvia the average day-length from May to September is 15.8 hours whereas in Kyrgyzstan only 13.7 hours. The HD value in Latvia was significantly lower compared to the corresponding field trial in Kyrgyzstan. In the field trials in

Kyrgyzstan the bred cultivars did not show differences in HD between years but there was a significant difference in response of six-rowed Russian landraces between the two years. It could be due to the fact that the advanced cultivars have less variation in response to year-to-year variation than compared landraces. In barley breeding programs one of the major problems is interaction between genotype x environment, when aiming to achieve the desired breeding targets in new cultivars to be used over large cultivation areas in different agro-environmental areas (Bleidere *et al.*, 2012; Dofing *et al.*, 1992). Among the overall studied material the two-rowed cultivars revealed a more stable response to year-to-year variation within the trial sites. In Kyrgyzstan the majority of barley cultivation areas are located in agronomically unfavorable conditions at high altitude provinces (up to 2500 m.a.s.l) often combined with low input agricultural practice (e.g. soil nutrition levels). Therefore, evaluation of the breeding material from different origin in different eco-environment conditions allow a better understanding the plant material and revealing new sources of useful adaptive traits for barley breeding. In barley breeding programs an establishment of various genetically diverse populations is one of the important key to solve the narrow genetic variation in working collection material (Ceccarelli *et al.*, 2001). In this study there were some cultivars ‘Saana’, ‘Sensis’, ‘Cecilia’ ‘Mari’, and ‘Mette’ (from Sweden and Latvia) which had attracting agronomic traits as candidate material for Kyrgyz barley breeding program for earliness, plant height (PH), SL and NGS. Other six-rowed cultivars ‘Jyva’, ‘Lavrance’ and landrace ‘Local 2’ can be used for earliness, SL, and NGS.

5 Conclusion, recommendation and future prospects

5.1 Conclusion

The genetic diversity of molecular markers, morphological and agronomical traits in spring barley potentially useful for Kyrgyz barley breeding is described in this thesis. The main conclusions are:

- ✓ A high genetic diversity exists in the 'The Farmers Mixture Populations' currently grown in Kyrgyzstan
- ✓ There is a significantly higher variation within the FMPs than among the FMPs.
- ✓ Based on molecular data there are no clear differentiation between FMPs and no distinct separation among morphological types or among the FMPs representing two different provinces of Kyrgyzstan.
- ✓ The origin of genotypes from FMPs could not be identified, with only a few exceptions, where individual plants were closely related to Kyrgyz bred cultivars.
- ✓ The SSR markers showed clear differentiation between FMPs and bred cultivars as well as differentiation from Asian and Russian landraces.

- ✓ The response of cultivars to different eco-geographic conditions depends on each agronomic traits.
- ✓ Evaluation of breeding material through different eco-geographic conditions allowed to identify superior cultivars which can be used for Kyrgyz barley breeding program as potential sources of various agronomic traits.

5.2 Recommendations and future prospects

The results obtained in our study provide a better understanding of genetic diversity level of farmers' material from different regions of Kyrgyzstan.

Based on the results of this thesis valuable germplasm of genetically distant barley, possessing desirable agronomic traits can be recommended to breeders for different breeding programs. Utilization of genes from cultivars and landraces characterized in this study can lead to improvement of barley cultivars in Kyrgyzstan.

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