Analysis of Genetic Diversity among Current Spring Wheat Varieties and Breeding for Improved Yield Stability of Wheat (Triticum aestivum L.)

Dissertation
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1 INTRODUCTION

1.1 Genetic diversity as a basis of crop improvement

Wheat is one of the most important cereal crops in the world. Its global consumption is close behind rice and maize. With the steadily growth of the world population, the demand for the food production is continually expanding (Lee et al., 1998; Hoisington et al., 1999). Especially, the demand for wheat is expected to increase faster than any other major crop such as rice and maize. To keep pace with the anticipated growth of human population, the predicted demand for the year 2020 varies between 840 (Rosegrant et al., 1995) and 1050 million tons (Kronstad, 1998). Given the fact that much existing arable land is decreasing due to urban and industrial development or natural erosion such as expanding deserts (Reif, 2004), genetic improvement of crops is considered as the most viable and sustainable approach to increase agricultural productivity (Tanksley & McCouch, 1997).

Effective crop improvement depends on the extent of genetic diversity in the gene pools. Over the past century, the achievements of plant breeding have contributed a lot to increase crop productivity and needs of societies by systemically genetic improvements with utilization efficiency of agricultural inputs (Warburton et al., 2002). However, these gains have often been accompanied by decreased genetic diversity within elite gene pools (Lee, 1998; Fernie et al., 2006). Although landraces have a diverse genetic base, they are therefore rarely integrated into the plant breeding programs due to their low productive performance. New varieties are usually derived from a set of genetically related modern high-yielding varieties. As a result, many landraces were continually replaced by modern wheat cultivars and crop improvement is still practiced in a narrow genetic base (Fernie et al., 2006).

It has been presumed that modern breeding practices with intensive selection leads inevitably to a loss of the genetic diversity in crops (Cluies-Ross, 1995; Tanksley & McCouch, 1997). Such reduction may have serious consequences. The vulnerability of crops against pests and diseases and the ability to respond
to changes in environmental conditions can be drastically influenced and threaten the sustained genetic improvement (Harlan, 1987; Tripp, 1996; Smale, 1996; FAO, 1996; Donini et al., 2000). This risk was brought sharply into focus in 1970 with the outbreak of Southern corn leaf blight (National Research Council, 1972). This disease drastically reduced corn yields in the United States due to the extensive use of a single genetic male sterility cytoplasm, which was associated with disease susceptibility. Other several server evidences occurred in India also in 1970s like epidemics of shoot fly (Atherigona spp.) and karnal bunt (Tilletia indica) (Dalrymple, 1986).

Reduction in diversity can be counterbalanced by introgression of novel germplasm. However, it should be noted that only a small proportion of the available genetic variation of the gene pools has been exploited for plant breeding so far (Frankel, 1977; Tanksley & McCouch, 1997; Fernie et al., 2006), but most of the exotic pools remain untapped, uncharacterized and underutilized (Alisdair et al., 2006). Therefore, the genetic variation provided by the current and expanded gene pools should be examined and harnessed for further crop improvement.

1.2 Evaluation methods of genetic diversity

Effective management and utilization of resources depends to a large extent on appropriate estimation of the material represented in the collection. Diversity can be generally characterized either by apparent diversity reflecting the different performance of crops across environments and management or by latent diversity referring to the genealogical and molecular measurements which are not necessarily expressed in crop performance (Smale et al., 2002). Several methods including pedigree records, biochemical markers and DNA marker can be performed to measure the latent diversity to quantify genetic diversity among genotypes (Cox et al., 1985; Karp et al., 1996).

1.2.1 Coefficient of parentage (COP)

The COP method is based on pedigree information and provides an indirect
measurement for the genetic diversity of cultivars by estimating the probability that alleles at a given locus are identical by descent. However, calculation of COP values has limitations because of the simplifying assumptions regarding relatedness of ancestors, parental contribution to the offspring, selection pressure, and genetic drift, which are generally not met (Cox et al., 1985; Cowen & Frey, 1987). Furthermore, pedigree records are not always available or detailed enough for such type of analysis, especially when large numbers of breeding lines or cultivars are being assessed (Parker et al., 2002).

### 1.2.2 Molecular markers

Diversity on a molecular level has been studied in plants for about three decades. The most comprehensive early studies were performed with biochemical markers such as isozymes and protein subunits (Hamrick & Godt, 1990; Weeden et al., 1994; Eagles et al., 2001) and provided many insights into population structure and breeding systems. Although these markers allowed large numbers of samples to be analyzed, only a limited number of loci could be scored. Furthermore, the comparison of samples from different species and laboratories were problematic (Buckler & Thornsberry, 2002).

In contrast, DNA markers offer quantitative views of genetic diversity among genotypes on the DNA level and have been widely accepted as potentially valuable tools to assess precisely genomic diversity in cereals, like wheat (Burkhamer et al., 1998; Eagles et al., 2001; Koebner, 2003), rice (Mackill et al., 1999), barley (Donini et al., 2001; Russell et al., 2000) and maize (Smith et al., 1997; Gauthier et al., 2002). In general, molecular markers can be classified into three categories based on their detection method: (1) hybridization-based such as restriction fragment length polymorphisms (RFLPs); (2) polymerase chain reaction (PCR)-based such as random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs), and (3) DNA chip and/or sequence-based such as single nucleotide polymorphisms (SNPs) (Gupta et al., 1999; Collard et al., 2005).
1.2.2.1 Restriction fragment length polymorphisms (RFLPs)

Among the various molecular markers, RFLPs were developed first and initially used to human genome mapping (Bostein et al., 1980). Later, these DNA marker technique was used in plant genome analysis including genome mapping (Weber & Helentjaris, 1989; Tanksley et al., 1989), variety identification (Vaccino et al., 1993) and assessing the level of genetic diversity and relationships within germplasm (Kim & Ward, 1997; Paull et al., 1989).

RFLPs refer to variation between genotypes in lengths of DNA fragments produced by restriction enzymes that cut genomic DNA at specific sites. The polymorphisms can arise either when mutations alter restriction sites, or result in insertions/deletions between these sites (Burr et al., 1983). The polymorphisms detected by RFLP technique compassed the recognition and cleavage by specific restriction enzymes and hybridization with a specific probe. Therefore, RFLPs have been shown the most reliable polymorphisms, which can be used for accurate scoring of genotypes. Further advantages of RFLP markers are the high level of information obtained by their co-dominant inheritance and their high level of reproducibility (Weeden et al., 1991; Helentjaris et al., 1985). However, several drawbacks limiting the use of RFLPs are: laborious, time-consuming, and low frequency of polymorphisms in crops especially in wheat (Bryan et al., 1997; Powell et al., 1996).

1.2.2.2 Random amplified polymorphic DNAs (RAPDs)

The polymerase chain reaction (PCR) technique (Saiki et al., 1988) facilitated the development of simple and low-cost molecular markers such as random amplified polymorphic DNAs (RADPs, Williams et al., 1990), amplified fragment length polymorphisms (AFLPs) (Vos et al., 1995), and simple sequence repeats (SSRs) (also known as microsatellites, Tauz & Renz, 1984).

RAPDs are based on amplification of DNA fragments by PCR using decamer primers homologous to random target sites in the genome (Williams et al., 1990). The polymorphisms revealed are either due to point mutations or insertions/deletions within the amplified region (Tingey & Deltufo, 1993).
RAPDs are much simpler and less laborious in comparison to RFLPs because they rely on a universal set of decamer primers without needs for prior sequence information and radioactive labeling of probes (Devos & Gale, 1992). However, since the natures of their random and short primer length, they cannot easily be transferred between species. They are mainly used as species-specific markers in diversity and phylogenetic studies, e.g. genome relationships in Triticeae (Joshi & Nguyen, 1993; Wei & Wang, 1995). Beside their dominant inheritance, their more general disadvantages are the sensitivity to the experimental conditions and a poor reliability and reproducibility (Karp & Seberg, 1996).

1.2.2.3 Amplified fragment length polymorphisms (AFLPs)

AFLPs are based on PCR amplification of restricted fragments generated by the combination of two specific restriction enzymes, the ligation of restriction site specific adapters and the use of adapter specific oligo-nucleotides with additional nucleotides at the 3’ end (Zabeau & Vos, 1993). The polymorphisms detected are due to modifications of restriction sites e.g. after point mutation (Vos et al., 1995).

AFLPs procedure involve three essential steps: (1) digestion of genomic DNA with two restriction enzymes (a low and a high frequent cutter), (2) ligation of adapter to the restriction ends, and (3) selective amplification of sets of restriction fragments by two successive PCR reactions using primers complementary to the restriction sites and adapter plus one to three additional nucleotides. Because this technique combines the reliability of the RFLPs technique with the power and ease of the PCR techniques (Jones et al., 1997), and exhibits intraspecific homology (Powell et al., 1996; Tohme et al., 1996), AFLP analysis is the most efficient method compared to RFLPs and RAPDs (Powell et al., 1996; Lin et al., 1996). However, the AFLPs method is technically difficult and expensive to set up, but it detects a large number of loci, reveals a great deal of polymorphisms and produces high complex DNA fingerprints, what is very useful in saturation mapping and for discrimination between varieties (Mohan et al., 1997; Jones et al., 1997).
1.2.2.4 Simple sequence repeats (SSRs)

Plant genomes contain large numbers of simple sequence repeats (SSRs) (also termed microsatellites). The core units of SSRs are usually one to five nucleotides, which are tandemly repeated and widely scattered at many different loci throughout the genome (Taute and Renz, 1984). These small repetitive DNA sequences provide the basis for a PCR-based, multi-allelic, co-dominant genetic marker system (Saghai-Maroof, 1994). Because the genome regions flanking the microsatellite are generally conserved among genotypes of the same species, SSR primers are designed matching unique flanking sequences, composed of short nucleotides, by which the microsatellite locus can be defined (Powell et al., 1996). Polymorphisms revealed by PCR-amplification are due to the variation of the number of repeats in a defined region of the genome (Morgante & Olivieri, 1993; Jones et al., 1993).

The utility of SSR markers is primarily deduced from their abundant distribution and hyper-variability in the whole genome (Morgante & Olivieri, 1993). Due to the existence of these hyper-variable regions, SSR markers exhibit a high power in distinguishing between closely related genotypes. Furthermore, the reproducibility of SSRs makes them interchangeable among different laboratories to produce consensus data.

However, the development of SSR markers is laborious and expensive. Through a public database to access primer sequences would maximize the use of microsatellites and reduce the development costs (Powell et al., 1996).

1.2.2.5 Single nucleotide polymorphisms (SNPs)

Single nucleotide polymorphisms (SNPs), referred to as single point mutations, have recently been developed into DNA markers, which offer high-throughput and automated genotyping approaches (Shi, 2001; Gupta et al., 1999). SNPs are highly abundant and distributed throughout the plant genomes such as maize (Edwards & Mogg, 2001; Tenaillon et al., 2001), barley (Kanazin et al., 2002) and in rice (Yu et al., 2002; Nasu et al., 2002). Various methods have been developed to genotype SNPs like pyrosequencing (Ahmadian et al., 2000),
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TaqMan (Livak, 1999), fluorescence energy transfer (Chen et al., 2000) or allelic-specific PCR (Drenkard et al., 2000). However, those using automated systems developed for high-throughput applications, which often require specific detection equipment, have high development costs and the marker assays generated are commonly not transferable between laboratories (Bundock et al., 2006). The development and use of allele-specific PCR-primers would be preferred due to its simplicity, low cost and reproducibility of genotyping SNP (Lee et al., 2004; Hayashi et al., 2004). By this approach, SNPs can be identified simply using allele-specific PCR primers designed that the 3’ terminal nucleotide of a primer corresponds to the site of the SNPs. The PCR-amplified products can be resolved on a standard agarose gel (Hayashi et

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Figure 1

Single nucleotide polymorphisms identified in a 263-nt segment of the maize stearoyl-ACP-desaturase gene (A Ching, unpublished data). The horizontal rows correspond to each of the 32 individuals sequenced. The vertical columns identify nine polymorphic sites, including one insertion/deletion (I/D) polymorphism. Four distinct haplotypes are shown. These four haplotypes can be unambiguously identified using only three SNPs, for example, those marked with an asterisk (*). The remaining SNPs provide redundant information. No two SNPs are sufficient to distinguish all four haplotypes (adapted from Rafalski 2002).
al., 2004; Lee et al., 2004). Through sequencing the PCR-amplified products from a number of diverse individuals, DNA polymorphisms can be detected in the most straightforward way compared to the other types of DNA markers based on the indirect detection of sequence-level polymorphisms including SSRs (Rafalski, 2002). Moreover, the PCR primers designed are either derived from the known DNA sequences of genes available from public GeneBank, or from expressed sequence tags (ESTs) (Rafalski, 2002). Therefore, the detection of SNPs provides the opportunity to uncover allelic variation directly within the sequences of genes or expressed sequences of candidate genes (Snowdon & Friedt, 2004). With the rapid development of public sequence databases in crop species, haplotype analysis is possible and more informative compared to individual SNP analysis (Figure 1) (Rafalski. 2002).

1.3 Lodging, its occurrence and types

Lodging of cereals is defined as permanent displacement of the culms from the upright position (Pinthus, 1973). It can result in buckling of the stem at a basal internode (stem lodging) (Figure 2) or in the rotation of the whole plant in the soil (root lodging) (Figure 3) (Zuber et al., 1999). Causes are usually a combination of wind and rain, but can be enhanced by different pathogens and pests affecting stems or roots (Keller et al., 1999) or by agronomic practices such as excessive fertilization and/or high seeding rates in wheat (Easson et al., 1993; Stapper & Fischer, 1990; Berry et al., 2000). Lodging can be a major constraint on yield potential in many crops, but it is of particular importance in the small-grain cereals, e.g. like wheat (Triticum aestivum L.) (Atkins et al., 1938; Easson et al., 1993), barley (Hordeum vulgare) (Baker et al., 1990; Travis et al., 1996), oat (Avena sativa) (Mulder, 1954; Murphy et al., 1958), corn (Zea mays) (Hondroyianni et al., 2000; Flint-Garcia et al., 2003), and rice (Oryza sativa) (Takahashi, 1960; Setter et al., 1997). At any stage of plant development (Atlins, 1938; Baker et al., 1990), lodging can occur and it is most detrimental at anthesis or in the early grain filling stage by reducing the number of kernels per ear and grain size (Laude & Pauli, 1956; Fisscher & Stapper, 1987; Briggs, 1990; Berry et al., 2004).
1.4 Effects of lodging on yield and quality of cereals

Grain yield reduction always accompanies lodging at which the degree of loss depends on the cultivar, growth stage and severity of lodging (Jedel & Helm, 1991; Easson et al., 1993; Fischer & Stapper, 1987). Several reports mentioned that lodging can reduce cereal production up to 20% (Briggs, 1990), 30% (Pinthus, 1973) or even 40% (Easson et al., 1993). Lodging can complicate harvesting and may cause deterioration in the milling and baking quality of the grains due to the increased moisture content of the grains and pre-harvest sprouting (Weber & Fehr, 1966; Kono, 1995). Furthermore, in lodged plants the contamination with mycotoxins produced by *Fusarium* species on the ears can
be significantly increased due to the humid atmosphere surrounding lodge crops (Langseth & Stabbetorp, 1996; Scudamore, 2000).

1.5 Factors affecting lodging

Lodging resistance is an important goal of cereal breeding. Lodging researches can be traced to 1930s for over a century and numerous efforts have been made to find and establish methods to assess lodging resistance so far. Most published studies before 1980 have been conducted on determining the correlations between morphological traits and lodging resistance (Clark & Wilson, 1933; Brady, 1934; Atkins, 1938; Sato, 1957; Jellum, 1962; Kohli et al., 1970; Stanca et al., 1979), whereas more recent publications have tried to established mechanical models for lodging resistance (Jezowski et al., 1987; Dolinski, 1990; Ennos, 1991; Crook & Ennos, 1993; Berry et al., 2006) or have focused on physiological and chemical components of the culms and their histological distribution (Dunn & Briggs, 1989; Kokubo et al., 1989; Zhu et al., 2004; Tripathi et al., 2003; Wang et al., 2006).

1.5.1 Plant height

Plant height is the major trait for the improvement of lodging resistance in cereal crops. A strong correlation between plant height and lodging has been reported for barley (Murthy & Rao, 1980, Stanca et al., 1979) and wheat (Atkins, 1938; Pinthus, 1967; Min et al., 2001). Since the 1960s, the introgression of dwarf genes has increased lodging resistance and grain yield (“green revolution”, Keller et al., 1999; Khush, 1999; Worland & Snape, 2001). Modern high-yielding cultivars are generally shorter with stronger straw, so a higher harvest index (Kelbert et al., 2004). Compared to high-growing plants, dwarfism increase lodging resistance through decreasing the centre gravity height of plant (Huang et al., 1988). According to Huang et al. (1988), plant height showed a significantly positive correlation with the centre gravity moment of plant \((r = 0.969)\) while lodging resistance index is significantly negative correlated with the height at centre of gravity \((r = -0.891)\). Hence, the history of lodging resistance
breeding was to some extent a history of dwarfism breeding (Wang et al., 1996). However, very short plants can reveal a decrease of biomass, high density of leaves, shrunken grains, premature senescence, aggravated diseases, etc. (Xiao et al., 2002). Therefore, continuous plant height reduction using dwarf genes may not be compatible with high yield (Berry et al., 2004). Moreover, lodging will still happen if the stem strength is not strong enough after dwarfing (Li et al., 1998; Min et al. 2001).

1.5.2 Stem characteristics

Lodging usually occurs when the stems bend or break at the basal internodes (Pinthus, 1973). Thus, the stem basal internode traits seem to be more important in comparison to other aerial traits of plants (Huang et al., 1988; Wang et al., 1996; Xiao et al., 2002). The stem basal internode traits comprised stem basal internode morphologies, anatomic characters, physiological factors, chemical ingredients, etc. (Wang et al., 1996).

1.5.2.1 Morphological characters

Under natural field conditions, lodging occurs in general sporadically. Thus, selection for lodging resistant cultivars is difficult in early generations of crops (Kelbert et al., 2004). Identification of easily measurable stem traits associated with lodging resistance may simplify the selection process and are a goal for cereal breeding.

The differences among lodging resistant and susceptible cultivars regarding various morphological characters of stems have been found in barley (Dunn & Briggs, 1989; Stanca et al., 1979), whereupon resistant cultivars exhibited shorter basal internodes, wider basal culm diameter and thicker culm walls compared to susceptible ones. Similarly, wider basal culm diameter and thicker culm walls associated with lodging resistant cultivars have been reported in wheat (Mukherjee et al., 1967; Shevchuk et al., 1981; Zuber et al., 1999; Tripathi et al., 2003) and oat (Jellum, 1962). Studies of Zuber et al. (1999) indicating that stem diameter explain 48% of the phenotypic variance of lodging
resistance while 50% of the phenotypic variance of lodging can be explained by stem weight cm$^{-1}$. Whether plants are resistant or susceptible to lodging is finally predicted by stem strength, implying mechanical elasticity and rigidity of the stem (Wang et al., 1996). The relation between lodging resistance and stem strength has been reported again in wheat (Atkins, 1938; Crook & Ennos, 1994; Pu et al., 2000) and barley (Clark & Wilson, 1933; Murthy & Rao, 1980). By path analysis, Pu et al. (2000) estimated the correlation between stem strength and lodging in a set of 11 high-yielding wheat varieties. The results indicated that an increase of one standard unit for stem strength was related to a decrease of 0.592 standard units for lodging index ($P_{xy} = -0.592$) in average, suggesting that plants with higher stem strength have a lower lodging index.

Focusing on the relationships between stem strength and stem basal internodes, several investigations have been performed. Xiao et al. (2002) observed that the stem diameter of the basal internodes was significantly correlated with stem strength from the milk to maturity stage ($r = 0.379$, 0.498 and 0.461), while the stem diameter of the upper internodes was not positively related to stem strength. More recently, Wang et al. (2006) found out that the thickness-diameter ratio showed significant correlation with stem strength at $r = 0.780$, whereas the stem wall thickness is significantly correlated with stem strength at $r = 0.551$. Similar results have been reported by Zhu et al. (2004). These two studies are comparable to the results of Huang et al. (1988) where the thickness-diameter ratio of stem showed a significant positive correlation with lodging resistance ($r = 0.681$). No significant correlation was observed between the stem wall thickness and the stem diameter versus lodging resistance in wheat, indicating that plants with high thickness-diameter ratio have high resistance to lodging (Huang et al., 1988).

However, dependent on the plant materials and crops deployed, different or contradictory results have been reported. For example, some authors did not find a significant correlation between stem diameter and lodging resistance in wheat (Atkins, 1938; Pinthus, 1967; Al-Qaudhy et al., 1988; Kelbert et al., 2004; Wang et al., 2006). In oats and barley, the negative correlation between the stem diameter and stem strength have been observed (Norden et al., 1970; Dunn et al., 1989).
1.5.2.2 Anatomical structure

The stem is one of the most important plant organs playing a key function in transportation, storage and mechanical support. Cereal stems are comprised of several nodes and internodes, mostly with pith cavities. The internodes close to the stem basis are generally shorter with a thicker stem wall compared to the upper internodes. The transverse section of internodes from the center to the outer layer is mainly composed of different tissues: pith, parenchyma, vascular bundles, sclerenchyma and epidermis. Vascular bundles are distributed within the transverse section in two circles: (1) small vascular bundles close to the epidermis embedded in sclerenchyma, which consists of fiber cells and (2) large vascular bundles, which are included in the parenchyma.

Up to now, many studies have been carried out to determine the relationship between anatomic stem characters and lodging resistance in wheat (Ford, 1979; Li, 1979; Cenci et al., 1984; Huang et al., 1988; Han et al., 1990; Wang et al., 1991; Wang et al., 1998; Zhu et al., 2004; Wang et al., 2006) and barley (Kokubo et al., 1989; 1991), respectively. Already in 1934, Brady reported that an increased number of vascular bundles is the most significant anatomical feature of the stem related to lodging resistance in wheat. According to Han et al. (1990), the percentage of mechanical tissue of stem and the number of vascular bundles mm\(^{-2}\) are closely correlated with lodging. However, Wang et al. (2006) revealed in wheat that the total number of vascular bundles mm\(^{-2}\) is negatively correlated with stem strength, whereas the number of large vascular bundles showed a positive correlation with stem strength \((r = 0.494)\). The same study mentioned a positive correlation between the percentage of sclerenchyma and stem strength \((r = 0.804)\). Similar results that the thickness of the sclerenchyma was responsible to lodging resistant have been reported for barley (Jezowski & El-Bassam, 1985; Dunn & Briggs, 1989). Studies on anatomical stem structure in rice also proposed that the difference of stem strength between normal and brittle stems was due to the differences of sclerenchyma and vascular bundles (Li et al., 2003). One explanation for this phenomenon can be that fiber cells extensively exist in vascular bundles and sclerenchyma, and it has been shown that fiber cells play a key role in
mechanical support of plants (Wang et al., 2006). Beside, in an interfascicular fiber mutant (ifl1) of Arabidopsis thaliana, Zhong et al. (1997) detected that a lack of interfascicular fibers is correlated with a dramatic change of stem strength. Stems of the mutant were not able to stand erected and were easily broken by bending in comparison to wild type stems.

1.5.2.3 Physiological factors and chemical ingredients

Many studies have indicated that the lodging resistance is not only due to morphological and anatomic stem characters, but well associated with physiological processes and chemical ingredients (Wang et al., 1996). The dry matter accumulated in the stem is mainly comprised of carbohydrates including monosaccharide, disaccharides and polysaccharides. The accumulation of dry matter, especially of polysaccharides the basis for cellulose and hemi-cellulose production, results in stem wall thickened and an increase of elasticity, which add up to an increase in stem strength (Wang et al., 1996). Several studies focusing on this issue have been performed and all indicated that the soluble carbohydrate content of the basal internodes of the stem contributed greatly to lodging resistance in wheat (Li et al., 1998) and rice (Sato, 1957; Takahashi, 1960; Matsuzaki et al., 1972; Taylor et al., 1999; Yang et al., 2001). According to Huang et al. (1988), the correlation between the carbohydrate content and lodging resistance can reach $r = 0.991$.

Plant cell walls possess of a strong fibrillous netted structure that provides mechanical support to cells, tissues, and the entire plant body (Li et al., 2003). Cellulose, hemi-cellulose and lignin as the main components of the cell wall, seem to have an intrinsic correlation with lodging resistance (Bernards & Lewis, 1998; Wang et al., 1996). For example, Taylor et al. (1999) and Jones et al. (2001) reported that lignin and cellulose content is related to stem rigidity. Huang et al. (1988) revealed that the lignin content of basal internodes of strong stems was higher compared to week stems. Kokubo et al. (1989) found a high correlation between the cellulose content of barley cell wall and maximum bending stress ($r = 0.93$).

However, whether the lignin content or cellulose content of stems is a
different conclusions have been reported regarding more recently studies. Zhu et al. (2004) and Jones et al. (2001) emphasized that the lignin content of stems is more important than the cellulose content for increasing the mechanical support. In contrast, Wang et al. (2006) reported that the cellulose content is more important in mechanical support in comparison to the lignin content \( r = 0.764 \) and \( r = 0.547 \), respectively).

In addition, several authors have investigated the relationship between chemical elements and molecules versus lodging resistance. The stem of wheat contains 2.3 - 4.6% silicon, which is mostly present in the epidermis of wheat culms and considered to contribute to lodging resistance (Li, 1979). Comparing the silicon contents between lodging resistant and susceptible wheat varieties, Gartner et al. (1984) observed a significantly higher silicon content in the epidermis and mechanical tissue of culms in the lodging resistant variety. Moreover, silicon of the cell wall was thought to contribute to mechanical strength in rice stems. Other chemical elements, like K, Ca and Mg are also associated with lodging resistance (Takahashi, 1995).

1.6 Evaluation methods and index for lodging

Several methods have been proposed and used to evaluate lodging. The most frequently used method is by visual ranking of naturally or artificially occurring lodging on a scale from 1 (all plants upright) to 9 (all plants flat) (Keller et al., 1999; Verma et al., 2005; Huang et al., 2006; McCartney et al., 2005). The ranking based on the fact that the degree and area of occurring lodging in the field directly reflect the lodging resistant level of crops. Different methods include the manually scoring of elasticity of the stem (Jezowski et al., 1987; Keller et al., 1999), the measurement of stem–breaking strength (Min et al., 2001; Wang et al., 1995) or testing the pushing resistance of the stem by specific instruments, like done for wheat (Xiao et al., 2002; Zhu et al., 2004; Wang et al., 2006), rice (Kashiwagi & Ishimaru et al., 2004; Terashima et al., 1992), barley (Kokubo et al., 1989) and corn (Fouere et al., 1995).

Vaidya et al. (1982) suggested that stem length × height per root weight and
breaking strength per height × shoot weight are the most suitable indexes of lodging resistance in wheat. Other indexes have been proposed like the moment of the gravity centre × load per fresh weight (Huang et al., 1988), height × aerial fresh weight per stem strength (Wang et al. 1995) and height × aerial fresh weight per root weight × stem strength (Pu et al., 2000). Because it is known that lodging is a complex trait covering many factors, up to now no method or index has been considered to be a reliable evaluation method for measuring and estimating lodging resistance.

1.7 Inheritance mode and chromosomal location of genes related to lodging

Studies concerning the mode of inheritance of lodging and related traits can be found in recent publications. Kohli et al. (1970) observed a transgressive segregation of traits related to lodging including stem strength, culm diameter and unit length weight in a segregating wheat F₂ generation and suggested a quantitative mode of inheritance of these three traits. Moreover, Li et al. (1998) analysed the general combining ability (GCA) and specific combining ability (SCA) of plant height at center of gravity, stem strength, pith diameter, stem wall thickness and mechanical tissues and indicated that these traits were controlled by genes with additive and non-additive gene effects. Stem fresh weight was controlled by non-additive genes, whereas small and large vascular bundles were controlled by genes with additive effects.

1.8 Quantitative trait loci (QTL) mapping

QTL analysis involved three main steps:
(1) Generation of mapping population;
(2) Genotyping and construction of a marker-based linkage map and
(3) QTL analysis combining linkage map and phenotypic values of traits.
1.8.1 Mapping population

Selection of parents is the key step for generation of mapping population. Most important is the discrimination of putative parental breeding lines, cultivars or landraces in at least one or even better in more traits of interest. Especially in self-pollination species such as most of the cereals, parental genotypes for mapping purposes should be almost homozygous. Several different types of mapping populations are used and can be classified into two categories according the genetic stability:

- Temporary segregation populations like F2 and backcross (BC) populations
- Fixed segregation populations like doubled haploid (DH) and recombinant inbred (RI) populations.

1.8.1.1 F2 population

The F2 population is common directly derived from F1 hybrids. Their major advantage is the easily done development in a short time independent of the reproduction system (self-pollination or cross-pollination) (Fang et al., 2001; Collard et al., 2005). However, F2 population comprise the maximum of heterozygousity (each locus will segregate in a 1:3 and, 1:2:1 ratio, respectively), where unfortunately dominantly inherited traits and molecular markers can not distinguished between dominant homozygous genotypes and heterozygous genotypes. Further disadvantage is that is not possible to conserved and proliferate a single F2 genotype without further segregation.

1.8.1.2 Backcross (BC) population

A BC population is derived from a cross between the F1 hybrid and one of the respective parents. This kind of population has similar advantages and drawbacks as F2 population. It also cannot be kept permanently. However, there is only a segregation ratio of 1 (homozygous): 1 (heterozygous) of each locus. Because the segregation ratio of the crossed F1 gamete directly reflects in BC population, the efficiency for mapping is higher compared to F2 population.
1.8.1.3 Doubled Haploid (DH) population

DH population can be produced through inducing the production of haploid plants by anther- or microspore-culture starting from the F1 hybrids. Doubling of chromosomes will happen spontaneously or after induction with colchicine, a “mitotic poison” originated from *Colchicum autumnale*, commonly known as autumn crocus. However, the production of DH population is only possible in species that are amenable to tissue culture, e.g. cereal species such as wheat, rice and barley (Fang et al., 2001). The major advantage of a DH population is that each single line is homozygous at each locus and can be multiplied and reproduced without genetic change occurring (Collard et al., 2005). Thus, why DH population is called permanent population permitting to conduct replicated trials across different locations and years. Furthermore, DH lines can be transferred between different laboratories for further linkage analysis (Paterson, 1996a; Yong, 1994). A major disadvantage of DH population is the different capability in tissue culture and therefore selection effects occurs, which may cause segregation distortion affecting later the precision of linkage between marker loci and thus the whole genetic map (Fang et al., 2001).

1.8.1.4 Recombinant inbred (RI) population

Population comprised of Recombinant inbreed lines (RILs) are developed by continued self-pollination of individuals starting from F2 plants by e.g. single seed descent (SSD) approach over several generations until almost all of segregating loci become homozygous. The major advantage of RILs just like DH-lines are that every line imply a unique combination of genomic segments from the ancestral parents in a homozygous manner and can be multiplied and reproduced without further segregation and change of genetic composition (Collard et al., 2005). RI populations as well as DH populations represent 'eternal' resources for QTL mapping and RI populations are also called permanent population (Yong, 1994; Paterson, 1996a). The major disadvantages of RI populations are the time consuming development and it may not be possible for each line to achieve homozygosity at every loci through limited
generations (in general six to eight) of self-pollination. A fact that decreases the efficiency for linkage map construction to some extent (Fang et al., 2001).

1.8.2 Linkage map construction

The construction of a linkage map essentially includes two steps: (1) grouping of linked markers into linkage groups, and (2) arranging the markers within each linkage group. Linkage between markers is usually determined by odds ratios, which represents the ratio of linkage versus no linkage (Fang et al., 2001). The ratio is more convenient expressed as the logarithm of the ratio and is called a logarithm of odds (LOD) value or LOD score (Risch, 1992). The commonly used threshold for the LOD value is ≥ 3.0 for statistical acceptance of linkage (Kosambi or Haldane function, Lu et al., 1998; Lincoln et al., 1993). A LOD value = 3.0 between two markers indicate that the linkage is 1,000 times more probable than no linkage (Collard et al., 2005). Length and distance of a linkage map are measured according to the frequency of recombination between two markers (Paterson, 1996a). Because the recombination frequency and the frequency of crossing-overs are not always linearly related (Kearsey & Pooni, 1996), mapping functions are required to convert recombination fraction into centiMorgans (cM). Therefore, two mapping functions are commonly used: Kosambi function (Kosamb, 1944) and Haldane function (Haldane, 1919). The main difference is that the Kosambi function assumes that recombination events influence the occurrence of adjacent recombination events, while Haldane function assumes no interference between crossover events (Hartl & Jones, 2001; Collard et al., 2005). Several software programmes can be used to perform the construction of linkage map. The most common ones are Mapmaker/EXP (Lincoln et al., 1993) and JoinMap (Biometris, Wageningen, The Netherlands, http://www.joinmap.nl).

1.8.3 Statistical methods for QTL mapping

The principle of QTL detection is to devide the mapping population into different genotypic groups based on genotypes at the marker locus and to determine
whether significant differences exist between groups with respect to the trait being measured. If the phenotypes between groups differ significantly, it indicated that the marker locus used to subdivide the population is linked to a QTL affecting the trait (Tanksley, 1993). Three main methods commonly used for QTL analysis are single-marker analysis, simple interval mapping (SIM) and composite interval mapping (CIM) (Liu, 1998; Tanksley, 1993).

1.8.3.1 Single-marker analysis

Single-marker analysis is the simplest method of QTL analysis and focus on individual associations between a single marker and the phenotypic characteristics. If an association is discovered, it is likely that there is a QTL affecting the trait linked to that marker locus (Zeng, 1994). Single-marker analysis based on analysis of variance (ANOVA) statistics, t-test and multiple regression analysis (Soller & Brody, 1976; Simpson, 1989; Rodolphe & Lefort, 1993). The most commonly used method is multiple regression analysis proposed by Rodolphe & Lefort (1993). Single-marker analysis by multiple regression analysis, e.g. for a DH population, is based on the following model:

\[ y_i = \mu + \sum_{j=1}^{m} b_j x_{ij} + \varepsilon_i \]  

\( y_i \) is the phenotypic value of the \( i^{th} \) individual;

\( i \) is individual; \( j \) is marker;

\( m \) is the number of the indicator variables;

\( \mu \) is the mean value; \( b \) is the partial regression coefficient of the phenotype \( y \) on the \( j^{th} \) marker;

\( x_{ij} \) is an indicator variable of the \( j^{th} \) marker in the \( i^{th} \) individual, taking a value of 1 if the \( i^{th} \) individual has the marker genotype \( j \) and 0 if otherwise;

\( \varepsilon_i \) is a residual error.

Regarding this model, the degree of correlation between each marker locus and phenotypic value is decided by the coefficient of partial regression. Generally, if the coefficient of partial regression reaches a determinated significance level, the QTL is indicated to be linked with the specific marker. Using this method, the
phenotypic variation explained by QTL can be determined by the coefficient of partial regression. However, using single marker analysis the genomic position of the QTL cannot be determined (Fang et al., 2001).

1.8.3.2 Simple Interval Mapping (SIM) method

SIM method is an extension of single-marker analysis and simultaneously analyses intervals between adjacent pairs of linked markers along several linkage groups (chromosomes), determines the likelihood profile of a QTL position at any particular point of each marker interval and calculates the LOD value (Lander & Botstein, 1989). So, SIM method can be considered as a statistical more powerful procedure compared to the single marker analysis (Liu, 1998). SIM procedure is also based on regression analysis. However, unlike single-marker analysis, SIM is based on the regression of phenotype and QTL instead of the regression of phenotype and marker locus. For example, regarding a DH population with only one QTL on a chromosome, Lander & Botstein (1989) proposed the following regression model to test for a QTL located on an interval markers \( j \) and \( j + 1 \):

\[
y_i = \mu + b^* x_i^* + \varepsilon_i
\]

(2)

\( b^* \) is the effect of the QTL;
\( x_i^* \) is an indicator variable of the putative QTL with a value of 0 or 1 with a likelihood depending on the genotypes of markers \( j \) and \( j + 1 \) and position being tested for the putative QTL.

Definitions of other parameters see (1)

Likelihood ratio (LR) test statistics uses the LOD score to estimate parameters and determine the significance of the regression:

\[
\text{LOD} = \lg \left[ \frac{L (b \neq 0)}{L (b = 0)} \right],
\]

(3)

where \( L (b=0) \) and \( L (b \neq 0) \) represent the maximum likelihood value (LOD≈0.217 LR) when \( b=0 \) and \( b \neq 0 \), respectively. If LOD exceeds a pre-defined threshold \( (b \neq 0) \), that is the effect of the putative QTL is not equal to 0, the existence of a
QTL can be deduced (Lander & Botstein, 1989). Using this method, the probable position of the QTL can be inferred by the supporting interval. The estimated locations and effects of QTL tend to be unbiased if there is only one QTL on a chromosome. However, if there are two closely linked QTL in one marker interval, a “ghost QTL” might appear between these two real linked QTL and the two real QTL will be hidden by the “ghost QTL” (Moreno-Gonzalez, 1992), which may result in a bias estimation of QTL and a decrease in the testing power (Fang et al., 2001).

1.8.3.3 Composite Interval mapping (CIM) method

CIM method is an extension of the simple interval mapping technique and is a combination of interval and multiple regression analysis (Zeng, 1994). The SIM method is modified by inclusion of additional markers as ‘cofactors’ in the regression model to get rid of the influence and background of other QTL to the target QTL interval (Fang et al., 2001). Regarding a DH population, testing of a QTL in a marker interval \((j, j + 1)\) by CIM, Zeng (1994) proposed the following regression model:

\[
y_i = \mu + b^* x_i^* + \sum_{k \neq j, j+1} b_k x_{ik} + \varepsilon_i
\]  

(4)

\(b^*\) is the effect of the putative QTL;

\(b_k\) is the partial regression coefficient of the phenotype \(y\) on the \(k^{th}\) marker;

\(x_i\) is an indicator variable of the putative QTL, taking a value 1 or 0 with probability depending on the genotypes of markers \(j\) and \(j + 1\) and position being tested for the putative QTL;

\(x_{ik}\) is a known coefficient for the \(k^{th}\) in the \(i^{th}\) individual, taking a value of 1 for the same marker genotype with one of the parents and 0 for the same marker genotype with the other.

Definitions of other parameters are the same as (1)

CIM method used the similar likelihood ratio test statistic compared to SIM method:

\[
LR = -2 \ln \left[ \frac{L(b = 0)}{L(b \neq 0)} \right],
\]

(5)

Compared to SIM, the threshold of the test statistic for the CIM is different.
Using multiple regression analysis in CIM, the test statistic is more or less uncorrelated for different interval, because the entire genome is tested for the presence of QTL rather than focusing on a particular interval by SIM (Zeng, 1994).

Because CIM method uses appropriate unlinked markers, which can partly account for the variation due to the unlinked QTL, and linked markers, which can reduce the variation resulting from linked QTL, in comparison to SIM method, the power of QTL detection is greatly improved by the CIM method (Jansen, 1996).

1.8.4 QTL mapping of agronomic traits in wheat

Up to now, numerous studies for QTL mapping have been carried out in many crop species. Particularly in cereals, QTL for major agronomic traits like yield and its components have been described in barley (Marquez-Cedillo et al., 2001; von Korff et al., 2006), in rice (Septiningsih et al., 2003; Takeuchi et al., 2003) and maize (Ho et al., 2002; Moreau et al., 2004).

In wheat, QTL for major agronomic traits have been extensively investigated so far. These traits includes grain filling time (Börner et al., 2002), maturity time (McCartney et al., 2005; Huang et al., 2006), heading date (Kato et al., 1999; Huang et al., 2003; Marza et al., 2006), seed dormancy, pre-harvest sprouting, grain color (Gross et al., 2002), and grain quality (Charmet et al., 2005; James et al., 2006; Perretant et al., 2000; Prasad et al., 2003; Cambell et al., 2001). Moreover, grain yield and yield components like grain weight, grain number and 1000-grain weight have been mapped by several studies (summarised in Table 1).

1.8.5 QTL mapping of lodging resistance and related traits in wheat

Focusing on QTL for lodging resistance and related traits, reports in many cereal crops like barley (Backes et al. 1995; Hayes et al., 1993; Tinker et al., 1996), rice (Champoux et al., 1995; Kashiwagi & Ishimaru, 2004), oat (De Koeyer et al., 2004) and maize (Flint-Garcia et al., 2003) are available.
Table 1 Summary of mapped QTL for yield and yield components in wheat (*Triticum aestivum* L.)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Chromosome</th>
<th>Population</th>
<th>Population type</th>
<th>Marker</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain yield</td>
<td>4A</td>
<td>CS/CS(kanto1074A)</td>
<td>RILs</td>
<td>RFLPs</td>
<td>Araki et al. (1999)</td>
</tr>
<tr>
<td>Grain</td>
<td>4A</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>50-grain</td>
<td>4A</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Plant height</td>
<td>4A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spikelet</td>
<td>4A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000-grain</td>
<td>3A</td>
<td>CNN/CNN (W13A)</td>
<td>RILs</td>
<td>RFLPs</td>
<td>Shah et al. (1999)</td>
</tr>
<tr>
<td>Grain</td>
<td>3A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant height</td>
<td>3A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grain yield</td>
<td>5A</td>
<td>CS(Capelle-esprez 5A)/CS (<em>T. spelta</em>5A)</td>
<td>RILs</td>
<td>RFLPs</td>
<td>Kato et al. (2000)</td>
</tr>
<tr>
<td>Grain</td>
<td>5A</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>50-grain</td>
<td>5A</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Spikelet</td>
<td>5A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grain</td>
<td>2D, 4A</td>
<td>W7984/Optata85</td>
<td>RILs</td>
<td>SSRs</td>
<td>Börner et al. (2002)</td>
</tr>
<tr>
<td>1000-grain</td>
<td>4A, 7D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grain</td>
<td>5A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant height</td>
<td>5A, 6A</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Grain yield</td>
<td>3A</td>
<td>Cheyenne/Wichita</td>
<td>RILs</td>
<td>RFLPs</td>
<td>Campbell et al. (2003)</td>
</tr>
<tr>
<td>1000-grain</td>
<td>3A</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Grain</td>
<td>3A</td>
<td></td>
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<tr>
<td>Plant height</td>
<td>3A</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Grain yield</td>
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<td>Renan/Récital</td>
<td>RILs</td>
<td>SSRs</td>
<td>Gross et al. (2003)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grain yield</td>
<td>2A, 2B, 3D</td>
<td>RI4452/AC Domain</td>
<td>DH</td>
<td>SSRs</td>
<td>McCartney et al. (2005)</td>
</tr>
<tr>
<td>1000-grain</td>
<td>2A, 3D, 4A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant height</td>
<td>2D, 4B, 4D</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Grain yield</td>
<td>1A, 1B, 2B</td>
<td>CS/SQ1</td>
<td>RILs</td>
<td>SSRs</td>
<td>Quarrie et al. (2005)</td>
</tr>
<tr>
<td>1000-grain</td>
<td>1A, 1B, 2B</td>
<td></td>
<td></td>
<td>RFLPs</td>
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<tr>
<td>Grain weight</td>
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<td>ACKarma/87E03S2B1</td>
<td>DH</td>
<td>SSRs</td>
<td>Huang et al. (2006)</td>
</tr>
<tr>
<td>1000-grain</td>
<td>2A, 3B, 6A</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Plant height</td>
<td>4B, 4D, 5D</td>
<td></td>
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<tr>
<td>Yield weight</td>
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<td>DH</td>
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<tr>
<td>Grain</td>
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<td></td>
<td></td>
<td>SSRs</td>
<td></td>
</tr>
<tr>
<td>Plant height</td>
<td>2B, 2D, 3B</td>
<td></td>
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</tbody>
</table>


Furthermore, QTL for lodging resistance have been reported in several studies on wheat summarised in Table 2.

**Table 2** Summary of mapped QTL for lodging resistance in wheat (*Triticum aestivum* L.)

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Population</th>
<th>Population type</th>
<th>Marker type</th>
<th>Reference</th>
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<tr>
<td>1B</td>
<td>Oberkulmer/Forno</td>
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<tr>
<td>2A</td>
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<tr>
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<td></td>
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<tr>
<td>4A</td>
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<td>5A</td>
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<tr>
<td>5B</td>
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<td></td>
<td>RFLPs</td>
<td></td>
</tr>
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<td>SSRs</td>
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</tr>
<tr>
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<td></td>
<td>RFLPs</td>
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<tr>
<td>1B</td>
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<td>DH</td>
<td>AFLPs</td>
<td>Marza et al. (2006)</td>
</tr>
<tr>
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<tr>
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</tr>
<tr>
<td>3D</td>
<td>RI4452/AC Domain</td>
<td>DH</td>
<td>SSRs</td>
<td>McCartney et al. (2005)</td>
</tr>
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<tr>
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2 OBJECTIVES

(1) SSR markers were used to describe and characterize the genetic diversity in a set of 69 spring bread wheat accessions from different geographical areas of the world but for the most part belonging to the European gene pools used for breeding purposes.

(2) QTL analysis of stem strength and related traits including stem diameter, culm wall thickness and pith diameter of basal internodes of wheat (*Triticum aestivum* L.) based on a DH population (cross CA9613 × H1488) to determine and analyse (i) genomic locations of the traits, (ii) markers associated with QTL, (iii) phenotypic effects, (iv) the homologous relationships among QTL and (v) to explore their utilization in wheat lodging resistance breeding by means of marker-assisted selection (MAS).
3 PUBLICATIONS

3.1 Quantitative structure analysis of genetic diversity among spring bread wheat (*Triticum aestivum* L.) from different geographical regions

Genetica (2006)

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Department of Plant Breeding, Research Centre for Biosystems, Land Use and Nutrition, Justus-Liebig University, Giessen, Heinrich-Buff-Ring 26-32, D-35392, Giessen, Germany

*Author for correspondence

3.1.1 Abstract

Genetic diversity in spring bread wheat (*T. aestivum* L.) was studied in a total of 69 accessions. For this purpose, 52 microsatellite (SSR) markers were used and a total of 406 alleles were detected, of which 182 (44.8%) occurred at a frequency of < 5% (rare alleles). The number of alleles per locus ranged from 2 to 14 with an average of 7.81. The largest number of alleles per locus occurred in the B genome (8.65) as compared to the A (8.43) and D (5.93) genomes, respectively. The polymorphism index content (PIC) value varied from 0.24 to 0.89 with an average of 0.68. The highest PIC for all accessions was found in the B genome (0.71) as compared to the A (0.68) and D genomes (0.63). Genetic distance-based method (standard UPGMA clustering) and a model-based method (structure analysis) were used for cluster analysis. The two methods led to analogous results. Analysis of molecular variance (AMOVA) showed that 80.6% of the total variation could be explained by the variance within the geographical groups. In comparison to the diversity detected for all accessions (*H*<sub>e</sub> = 0.68), genetic diversity among European spring bread wheats was *H*<sub>e</sub> = 0.65. A comparatively higher diversity was observed between wheat
varieties from Southern European countries (Austria/Switzerland, Portugal/Spain) corresponding to those from other regions.

**Key words:** genetic diversity, microsatellites, spring bread wheat, *Triticum aestivum* L., SSRs, quantitative structure analysis

### 3.1.2 Introduction

Effective crop improvement depends on the existence of genetic diversity. Trends concerning the loss of genetic diversity due to modern breeding practice have been reported by several studies (Russell et al., 2000; Roussel et al., 2004; Fu et al., 2005). Therefore, it seems necessary to understand the levels and distribution of genetic diversity in existing crop gene pools, as a basis for developing strategies of resource management and exploitation. Considering broadening the genetic base of crops for the maintenance of substantial breeding progress, exotic germplasm has shown to be a valuable resource, especially basic materials possessing specific agronomic traits such as disease and pest resistance. Furthermore, for incorporating exotic germplasm into respective breeding programmes, the genetic relationship between exotic accessions and adapted cultivars should be studied.

Molecular markers have been shown to be reliable tools to assess genomic diversity. However, some of the marker systems, such as restriction fragment length polymorphism (RFLP) (Botstein et al., 1980) and random amplified polymorphic DNA (RADP) (Williams et al., 1990) have been of limited use for crop plants due to their low polymorphism, particularly in self-pollinating species with a narrow genetic basis, such as bread wheat (Sharam et al., 1983). On the other hand, simple sequence repeats (SSRs) (Tautz et al., 1989) have been widely exploited in wheat due to their high level of polymorphism, co-dominant inheritance and equal distribution in the wheat genome (Röder et al., 1995; Parker et al., 2002). Up to now, besides their application for identifying genotypes and detecting genetic diversity (Plaschke et al., 1995; Prasad et al., 2000), SSRs have been used for the characterization of the genetic integrity of gene bank accessions (Börner et al., 2000), the genetic differentiation caused
by selection (Stachel et al., 2000), and temporal changes in genetic diversity (Donini et al., 2000; Christiansen et al. 2002; Roussel et al., 2004). Moreover, comparisons of genetic diversity among gene pools from different geographical origin in Europe or worldwide have been carried out by this approach (Roussel et al., 2005; Röder et al., 2002; Huang et al., 2002).

For statistical data analysis and presentation, the UPGMA clustering is commonly used as a standard procedure. More recently, a model-based clustering method, the so-called structure analysis was developed by Pritchard et al. (2000), and first used for association studies in Human genetics (Pritchard and Przeworski, 2001; Rosenberg et al., 2002). This method uses Bayesian clustering and allows to characterize populations (or groups) by allele frequencies at each locus, and individuals in the samples can be assigned to one or more population(s) or group(s) based on probability. Therefore, structure analysis is considered as a more suitable approach to fine statistical inference than the distance-based UPGMA clustering (Pritchard et al. 2000). This method has recently been applied for structural analysis of populations or the identification of genetically distinct groups in crop species, such as rice (Jain et al., 2004; Lu et al., 2005), maize (Liu et al., 2003), barley (Ordon et al., 2005) and wheat (Maccaferri et al., 2005).

In the present study, SSR markers were used to characterize the genetic diversity in a set of 69 spring bread wheat accessions selected on the basis of their diverse origin from different geographical areas of the world. Cluster analysis was performed by, both, the genetic distance-based and the model-based methods. Finally, genetic relationship and diversity levels were analysed to describe and characterise the European gene pools for breeding purposes.

3.1.3 Materials and methods

3.1.3.1 Plant materials

A total of 69 spring bread wheat (Triticum aestivum L.) accessions including 66 cultivars and three landraces out of a German evaluation program (EVAII) were used for this study. Among them, 56 accessions originated from different
European countries like Austria and Switzerland (A/CH), Czech Republic (CZ), France, Germany and Netherlands (F/G/NL), Norway and Sweden (N/S), Portugal and Spain (E/P), the United Kingdom (UK), and 13 varieties originated from North America (Canada, USA), South America (Argentina, Brazil), and East Asia (China, Japan). Nyubay and Arurakomugi (from Japan) and Wangshuibai (from China) represent landraces (Table 3).

### Table 3

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### 3.1.3.2 DNA extraction and SSR analysis

Seeds were sown and grown in pots in the greenhouse. For each accession fresh leaf material of five plants were pooled and bulk genomic DNA was extracted according to a standard CTAB method (Doyle and Doyle, 1990). Fifty-
Table 4 Chromosomal location, number of alleles, number of rare alleles and polymorphism information content (PIC) values per locus for 52 microsatellite loci in two sets of data: all 69 accessions (aa) and 56 European accessions (ea), respectively.

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<td>0</td>
</tr>
<tr>
<td>wmc601</td>
<td>2DL</td>
<td>14</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>wmc167</td>
<td>2DL</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>xgwm341</td>
<td>3DL</td>
<td>11</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>wmc418</td>
<td>3DL</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>wmc52</td>
<td>4DL</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>wmc331</td>
<td>4DL</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>wmc233</td>
<td>5DS</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>wmc215</td>
<td>5DL</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>wmc97</td>
<td>5DL</td>
<td>7</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>wmc161</td>
<td>5DL</td>
<td>8</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>barc196</td>
<td>6DS</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>barc96</td>
<td>6DL</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>wmc273</td>
<td>7DL</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

*The chromosome location of SSR markers according to Somers et al (2004)
two microsatellite markers representing at least one SSR marker for each chromosome were selected from the wheat microsatellite consensus map published by Somers et al. (2004) (Table 4). PCR was carried out with the M13–tailing technique described by Berg and Qlaisen (1994). For this method, the fluorochrome-labeled universal M13 primer sequence 5'AGGGTTTTCCAGTCACGACGTT3' (MWG Biotech, Ebersberg, Germany) was added to the 5' end of each forward primer. After the first round of amplification, the PCR fragments were subsequently amplified by the labeled universal primer. The PCR reactions were performed in a volume of 25µl containing 1x PCR Buffer with 1.5 mM MgCl₂, 200 µM of each dNTP (Promega, Madison, WI, USA), 0.2 pmol forward primer, 2 pmol reverse primer and 1.8 pmol M13 oligonucleotide (IRD700- or IRD 800-labelled), 50 ng template DNA and 0.5 units Taq polymerase (Eppendorf, Westbury, NY, USA). PCR amplification reactions were carried out in a thermocycler model 9700 (Perkin-Elmer, Norwalk, CT, USA). The PCR reaction mixture was denatured at 95°C for 3 min, followed by 35 cycles of 94°C for 1 min; either 50°C, 55°C, or 60°C (depending on the primer pair) for 1 min, 72°C for 2 min with a final extension step of 72°C for 5 min. Separation of SSR amplified products were visualized using an automated laser fluorescence sequencer LI-COR 4200 (LI-COR Biosciences, Lincoln, NE, USA).

3.1.3.3 Statistical analysis

SSR profiles were scored reflecting either the presence “1” or absence “0” of bands. The sizes of fragments were determined in compassion to a molecular weight standard using the software package RFLP Scan 2.1. Genetic similarity between pairs of accessions was estimated according to DICE similarity coefficient (Dice, 1945) based on the proportion of shared alleles using SIMQUAL (similarity of qualitative data) routine by software NYSTS-pc 2.0 (Rohlf, 2000),

\[ G_{\text{D}ic} = \frac{2a}{2a + b + c}, \]

where a refers to alleles shared between two varieties, and b and c refer to alleles present in either one of the two varieties.
Variability at each locus was measured using polymorphism information content (PIC),

$$\text{PIC} = 1 - \sum_{i=1}^{n} p_{ij}^2$$

where $n$ is the number of alleles of locus $j$ and $p_{ij}$ is the frequency of the $i^{th}$ allele of locus $j$ (Botstein et al., 1980).

Correlations and regression analyses were performed using SAS 6.0 (SAS Institute, Cary, NC, USA).

Whether the number of SSR markers deployed in this study will provide sufficient information on allele diversity in the whole data set of 69 genotypes and the European subset of 56 genotypes, respectively, or not was evaluated by calculating the average coefficient of variation (CV) of the PIC value. Therefore CV was estimated for each SSR marker by bootstrapping (Efron, 1986) where SSR markers were submitted to 1,000 samplings one by one with replacement. This procedure was repeated with a continuous growing number of markers until the total number of 52 SSR markers was reached. The mean CV was plotted against the number of SSR markers.

Cluster analysis was carried out by software POPGENE version 1.32 (Yeh et al., 1999) using the UPGMA method. Nei’s unbiased genetic distance (Nei, 1978) was used to calculate the pair-wise genetic distance among all accessions. Dendrograms were visualized with the TreeView programme (Page, 1996).

Model-based cluster analysis was performed by the software STRUCTURE version 2.0 (Pritchard et al., 2000), which is designed to identify the population structure by a set of allele frequencies at each locus and assign individuals to populations. The number of presumed populations ($K$) was set from 2 to 10, and each was repeated three times. For each run, burn-in and iterations were set to 50,000 and 100,000, respectively, and a model allowing for admixture and correlated allele frequencies. When alpha was constant, the run with maximum likelihood was used to assign individual genotypes into groups. Within a group, genotypes with affiliation probabilities (inferred ancestry) $\geq 80\%$ were assigned to a distinct group, and those with $< 80\%$ were treated as “admixture”, i.e. these genotypes have a mixed ancestry from parents belonging to different gene pools or geographical origin.
Analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was performed to test the significance of the partitioning of genetic variance between and within the detected groups. AMOVA was carried out using the software ARLEQUIN version 2.0 (Schneider et al., 2002). Relationships among groups were inferred using UPGMA clustering method based on Nei’s unbiased genetic distance (Nei, 1978) with POPGENE version 1.32 (Yeh et al., 1999).

Genetic differentiation between groups was quantified with $F_{st}$ (Slatkin, 1995) values based on all 52 microsatellite loci using software FSTAT version 2.9.3 (Goudet, 2001). $F_{st}$ may be interpreted as the correlation between allele frequencies of different individuals in the same population under an infinite allele model (IAM) (Weir & Cockeberham, 1984), and it thought to be more appropriate for recently diverged populations (Olsen and Schaal, 2001). A significance test of population differentiation (pairwise $F_{st}$) was performed by randomizing genotypes among samples to obtain the log-likelihood G-statistics (Goudet et al., 1996). Significance tests of correlations were performed by bootstrapping over loci with a 95% nominal confidence interval. The sequential Bonferroni correction was implemented for the multiple tests (Rice, 1989).

In order to specify the level of diversity between wheats of the European germplasm, statistical descriptive parameters like number of allele per locus or allelic richness ($A$), the number of rare alleles per locus and Nei’s average gene diversity ($H_a$) (Nei’s 1978) were calculated for each European subgroup. Considering that the observed number of alleles in a sample set is highly dependent on the sample size, allele rarefaction method was performed to estimate unbiased $A$ by software FSTAT version 2.9.3 (Goudet, 2001) as suggested by El Mousadik and Petit (1996).

The number of rare alleles per locus of each group was estimated following Roussel et al. (2004) with minor modifications. The ratio was calculated as the total expected number of alleles per locus by rarefaction divided by the total observed of alleles per locus for each group before multiplying the observed number of rare alleles per locus by this ratio.

Average gene diversity ($H_a$) was estimated as mean genetic diversity over loci and adjusted for the sample size according to Nei (1978):
where \( p_{ij} \) is the frequency of the \( i^{th} \) allele of locus \( j \), \( n_i \) is the number of genetic loci, and \( n_a \) is the number of accessions.

### 3.1.4 Results

#### 3.1.4.1 SSR polymorphisms and genetic diversity

Fifty-two SSR markers, covering all 21 chromosomes of the hexaploid wheat genome with one to four SSR markers per chromosome were used to characterise the genetic diversity of 69 spring bread wheat accessions (Table 4). All loci used in this study were multiallelic, ranging from two (\( wmc233 \) and \( wmc245 \)) to 14 (\( wmc601, wmc754 \) and \( wmc326 \)) with an average of 7.81 ± 3.28 alleles per locus. The largest number of alleles per locus occurred in the B genome (8.65 ± 3.19) in comparison to the A (8.43 ± 2.87) and D genomes (5.93 ± 3.42). In total, 406 alleles were detected in the whole set of 69 accessions (Table 5), of which 182 (44.8%) occurred at a frequency of < 5% and are considered as rare alleles varying from 0 (\( xgwm129, wmc307, wmc245, wmc233 \) and \( barc196 \)) to 9 (\( wmc326 \)). The PIC values of the 52 SSR loci ranged from 0.24 (\( wmc47 \)) to 0.90 (\( xgwm120 \)) with an average of 0.68 ± 0.16. The highest PIC value for all accessions was identified for the B genome (0.71 ± 0.17) compared to the A (0.68 ± 0.13) and the D genomes (0.63 ± 0.15). Focusing on the European subset of 56 accessions, 320 alleles were detected including 100 rare alleles (Table 4). Regarding the number of alleles per locus the European subset showed slightly lower values in comparison to the whole set of analysed accessions (Table 5). In the subset of European wheats the PIC value for the A genome has been found to be equal to the PIC of the D genome, while the B genome again had the highest PIC in European accessions. Further on, most of the genetic loci showed a higher diversity for all accessions in comparison to the European subset alone. Especially the SSR loci \( wmc51, wmc245, wmc601, wmc167, wmc52, wmc233 \) and \( barc196 \) showed fewer
alleles in the European accessions, whereas the diversity in the whole set and the European wheats were similar for SSRs wmc418 and wmc215 (Table 4). Additionally, a significant correlation between the PIC values and the number of alleles were observed in the whole set of 69 genotypes ($r = 0.82$, $P < 0.0001$) and for the European subset of 56 genotypes ($r = 0.71$, $P < 0.0001$), respectively. In order to specify the effect of the sample size on the accuracy of the estimated genetic diversity, CV of the PIC value estimated on 1,000 bootstrap re-samples revealing an asymptotic curve (Figure 4) where an increasing number of loci led to decreased CV (CV = 3.2% and 3.42% in the whole data set of 69 accessions and the European subset of 56 accessions, respectively)

Table 5 Number of alleles, number of rare alleles, number of alleles/locus and mean PIC value cross 52 microsatellite loci in the A, B and D genomes in all accessions (aa) and in European accessions (ea).

<table>
<thead>
<tr>
<th>Item</th>
<th>Accessions</th>
<th>Genome</th>
<th></th>
<th></th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loci</td>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>D</td>
</tr>
<tr>
<td>Number of alleles</td>
<td>aa</td>
<td>118</td>
<td>199</td>
<td>89</td>
<td>406</td>
</tr>
<tr>
<td></td>
<td>ea</td>
<td>89</td>
<td>153</td>
<td>78</td>
<td>320</td>
</tr>
<tr>
<td>Number of rare alleles</td>
<td>aa</td>
<td>57</td>
<td>93</td>
<td>32</td>
<td>182</td>
</tr>
<tr>
<td></td>
<td>ea</td>
<td>31</td>
<td>48</td>
<td>21</td>
<td>100</td>
</tr>
<tr>
<td>Number of alleles/Locus</td>
<td>aa</td>
<td>8.43 ± 2.87</td>
<td>8.65 ± 3.19</td>
<td>5.93 ± 3.42</td>
<td>7.81 ± 3.28</td>
</tr>
<tr>
<td></td>
<td>ea</td>
<td>6.36 ± 2.80</td>
<td>6.65 ± 2.40</td>
<td>5.20 ± 2.93</td>
<td>6.15 ± 2.69</td>
</tr>
<tr>
<td>Mean PIC value</td>
<td>aa</td>
<td>0.68 ± 0.13</td>
<td>0.71 ± 0.17</td>
<td>0.63 ± 0.15</td>
<td>0.68 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>ea</td>
<td>0.63 ± 0.16</td>
<td>0.66 ± 0.18</td>
<td>0.63 ± 0.17</td>
<td>0.64 ± 0.17</td>
</tr>
</tbody>
</table>

Figure 4 Coefficient of variation of the PIC values among all accessions estimated by bootstrap analysis for subsamples with an increasing number of SSR loci.
3.1.4.2 Genetic similarity and relatedness among accessions

Pair-wise genetic similarity ($G_{\text{Dice}}$) among all accessions varied widely from 0.08 (Coa vs. Mellissos, and Dragon vs. Nyubay) to 1.00 (Amazonas vs. Avle). The average $G_{\text{Dice}}$ within accessions was estimated at 0.31 in comparison to an average $G_{\text{Dice}}$ of 0.35 within the European subset (data not shown).
Distance-based UPGMA cluster analysis divided all 69 accessions into five main groups (Figure 5). Within group 1, six accessions were differentiated into two subgroups comprising, two Portugal varieties and Universal (Argentina) on the one hand and three Chinese varieties on the other hand. The larger group 2 and group 3 comprised almost all European accessions including the varieties AC Reed (Canada) in group 2 and IDO232 and Transec (USA) in group 3. Group 2 mainly contains varieties from the Czech Republic (5) and most of the varieties from Portugal (6). All the Czech varieties exclusively cluster together in a subgroup within group 2, whereas the remaining varieties are distributed to further subgroups. Group 3 comprises 39 accessions from France, Germany, the Netherlands, and UK (57%) and contains four subgroups (a, b, c and d). In subgroup (a) five UK varieties clustered together along with one French variety, whereas three German and two Dutch varieties generated further subgroups. Subgroup (b) consists of 21 accessions including Swedish (6) and German (10) varieties along with one Austrian and four varieties from Switzerland and the Netherlands, respectively. Subgroup (c) includes all Norwegian varieties and one Swedish variety, whereas two US varieties along with B5769 (Switzerland) are placed in subgroup (d). Group 4 contains further the two Swiss varieties on the one hand and two Brazilian varieties along with the Argentinean Eureka F.S.C. on the other hand. Three landraces are well separated from the remaining cultivars representing group 5.

STRUCTURE software was used to perform model-based cluster analysis. In a range of simulated runs for different $K$ values from 2 to 10 (presumed number of populations), the most appropriate number of groups ($K$) was identified at $K = 9$ with a constant alpha ~ 0.03 and the natural log probability of the data which is proportional to the posterior probability was maximized (-4412.0). The 69 accessions were assigned into nine genetically distinct groups, except 22 accessions identified as admixtures having 37.5 - 78.3% shared ancestry with one of the major groups (Figure 6). In comparison to UPGMA clustering, structure analysis led to analogical results. Groups VIII and IX identified by the structure analysis were identical to group 4 and 5 of UPGMA clusters. Three landraces assembled in group IX shared an average of 95% ancestry with each other. In group VIII, two Brazilian varieties shared 98.7% ancestry, whereas
Figure 6 Graph showing the proportion of shared ancestry among the 69 spring bread wheats based on 52 SSR markers using model-based method by structure (Pritchard et al, 2000). The symbol “•” represent admixtures.
other accessions were identified as admixtures, showing only 66% mean shared ancestry with the major group. Groups III and IV are conform to group 2 of UPGMA clustering. The Czech varieties present groups III showing more than 93% mean shared ancestry were clearly separated from the accessions in group III. Within group III, nine out of 10 accessions had an average of 88.5% shared ancestry, only the variety Mondego (Portugal) was identified as an formed group II and was comparable to one subgroup of group 1 according to UPGMA clustering with an average of 96.4% shared ancestry. The remaining three accessions of group 1 (UPGMA clustering) together with two USA accessions and variety Jordao (Spain) from group 3 and group 2, respectively, reveal nearly 90% shared ancestry and were placed into group I (structure). All accessions in the groups V, VI and VII represented European varieties, corresponding to group 3 (UPGMA clustering). Hereby group V corresponds to subgroup (a) including two German varieties, two UK varieties and the Dutch cv. Minaret with an average shared ancestry of 95.2%, whereas the other five accessions with only 60% mean shared ancestry were identified as admixtures. Group VI correspond to subgroup (b), where out of the 22 accessions, 13 mainly including varieties Swedish and German ones as well as the Dutch cv. Baldus, showed 94% shared ancestry, whereas nine accessions were identified as admixtures (56% shared ancestry). Group VII corresponds to subgroup (c) comprising two Norwegian cultivars with 94.5% shared ancestry and the accession Brakar with only 56.4% shared ancestry.

3.1.4.3 Relevance of geographical origin for genetic variation

By investigating the genetic variation among spring wheat accessions, all samples (69 accessions) were assigned to 10 geographical pools, whereas the European accessions (56 genotypes) were differentiated into six pools. AMOVA test was carried out at two hierarchical levels, between and within 10 geographical groups, and between and within European groups, respectively (Table 6). On both hierarchical level, the variation is highly significant, but the variation within groups had a major effect amounting to 80.6% and 84.4% of the total variation for the whole set and the European accessions, respectively.
3.1.4.4 Relationship and diversity between six European geographical groups

For a better understanding of genetic variation within the European germplasm, genetic diversity and differentiation was investigated for the geographical groups including Austria/Swiss (A/CH), Czech Republic (CZ), Portugal/Spain (E/P), France/Germany/Netherlands (F/G/NL), Norway/Sweden (N/S), and the United Kingdom (UK). A diagram was generated based on Nei’s unbiased genetic distance by UPGMA to show the genetic relationships between these groups and was presented in Figure 7, which showed two broad groups. One comprises CZ and E/P, whereas the accessions from A/CH, F/G/NL, N/S and UK cluster together in one other group. Pairwise $F_{st}$ among the six groups of European germplasm, five showed a significant differentiation. Group F/G/NL showed the significant differentiation to the groups A/CH, E/P and UK, whereas A/CH and E/P were significantly different to UK, respectively. The mean genetic diversity between European accessions is estimated at 0.65 ($H_e$) compared to $H_e = 0.68$ for all accessions. Regarding each geographical group, a higher diversity was found within A/CH ($H_e = 0.63$) and E/P ($H_e = 0.62$), whereas a lower diversity was observed in the pools F/G/NL ($H_e = 0.55$) and N/S ($H_e = 0.51$), and especially within UK ($H_e = 0.42$) and CZ ($H_e = 0.37$). The of alleles number per locus are same ordered starting with the highest values within A/CH (2.92), E/P (2.88) and F/G/NL (2.56), followed by N/S (2.38), UK (2.01) and CZ (1.84). Analogically, the highest percentage of rare alleles per locus was detected among the E/P wheats (27.5%), whereas the lowest was observed within the CZ group (3%) (Figure 8 and 9).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Sum of squares</th>
<th>Variance</th>
<th>% Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>All accessions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among the groups</td>
<td>9</td>
<td>680.6</td>
<td>7.1***</td>
<td>19.4</td>
</tr>
<tr>
<td>Within the groups</td>
<td>59</td>
<td>1727.3</td>
<td>29.3***</td>
<td>80.6</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>2407.9</td>
<td>36.3</td>
<td></td>
</tr>
<tr>
<td>European accessions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among the groups</td>
<td>5</td>
<td>386.3</td>
<td>5.4***</td>
<td>15.6</td>
</tr>
<tr>
<td>Within the groups</td>
<td>50</td>
<td>1460.1</td>
<td>29.2***</td>
<td>84.4</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>1846.5</td>
<td>34.6</td>
<td></td>
</tr>
</tbody>
</table>

*** Significant level at $P < 0.001$. 

Table 6 Analysis molecular of variance (AMOVA): effect of geographical groups
Figure 7 Relationship between six European geographical groups based on Nei’s unbiased genetic distance (1978). A/CH = Austria/Switzerland; CZ = Czech Republic; E/P = Spain/Portugal; F/G/NL = France/Germany/Netherlands; N/S = Norway/Sweden; UK = the United Kingdom

Table 7 Matrix of $F_{st}$ (below) and Nei’s unbiased genetic distance (above) between the six groups particularly originated from Europe, calculated for 52 microsatellite loci

<table>
<thead>
<tr>
<th>Group</th>
<th>A/CH</th>
<th>E/P</th>
<th>UK</th>
<th>CZ</th>
<th>N/S</th>
<th>F/G/NL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/CH</td>
<td>-</td>
<td>0.293</td>
<td>0.468</td>
<td>0.420</td>
<td>0.344</td>
<td>0.212</td>
</tr>
<tr>
<td>E/P</td>
<td>0.057</td>
<td>-</td>
<td>0.724</td>
<td>0.439</td>
<td>0.480</td>
<td>0.487</td>
</tr>
<tr>
<td>UK</td>
<td>0.161*</td>
<td>0.244*</td>
<td>-</td>
<td>0.640</td>
<td>0.417</td>
<td>0.239</td>
</tr>
<tr>
<td>CE</td>
<td>0.165</td>
<td>0.179</td>
<td>0.343</td>
<td>-</td>
<td>0.627</td>
<td>0.427</td>
</tr>
<tr>
<td>N/S</td>
<td>0.130</td>
<td>0.186</td>
<td>0.211</td>
<td>0.310</td>
<td>-</td>
<td>0.226</td>
</tr>
<tr>
<td>F/G/NL</td>
<td>0.065*</td>
<td>0.179*</td>
<td>0.100*</td>
<td>0.211</td>
<td>-</td>
<td>0.111</td>
</tr>
</tbody>
</table>

* Significant level at $P < 0.05$

Figure 8 Nei’s average gene diversity ($H_e$) (Nei, 1978) for each European geographical group based on 52 microsatellite loci. A/CH = Austria/Switzerland; CZ = Czech Republic; E/P = Spain/Portugal; F/G/NL = France/Germany/Netherlands; N/S = Norway/Sweden; UK = the United Kingdom.
3.1.5 Discussion

Bread wheat (Triticum aestivum L.) is an allohexaploid (2n = 6x = 42) plant and comprises three sub-genomes: A, B and D. The diversity levels of the sub-genomes have been shown to be different: the highest PIC is usually reported for the B genome followed by the A and D genomes (Huang et al., 2002; Stachel et al., 2000). The highest genetic diversity in the present study was found in the B-genome (PIC = 0.71) as compared to PIC values of 0.68 and 0.63 for the A and D genomes, respectively (Table 5). These results correspond to those of Huang et al. (2002) who estimated somewhat higher but similarly diverse PIC values for the B (0.81), A (0.78) and D (0.73) genomes. The fact that the latter values are higher is probably due to a larger number of diverse wheat types (almost 1,000 accessions) originating from all over the world. On the contrary, Stachel et al. (2000) observed lower PIC values in 60 accessions originating from three European countries, i.e. Austria, Germany and Hungary. Similarly,
this can be explained by a lower geographical diversity of the genotypes investigated in their just like our study. Among 559 French varieties (Roussel et al., 2004) higher PIC value was detected in the D genome than in the A genome, but still the highest PIC value was observed in the B genome. This finding can be explained by the larger number of landraces (62 vs. 559 total genotypes) in their study. By comparing the difference of diversity between the A and D genomes in landraces (229 accessions) and modern varieties (111 accessions), respectively, Hao et al. (2006) found that more diversity exists in the D genome of landraces compared to the A genome, whereas the diversity in the A genome is higher than in the D genome in modern varieties. However, the highest genetic diversity was detected in the B genome. This observation supported our assumption mention above. Furthermore, a higher difference of diversity in the D genome between modern varieties and landraces was reported in comparison to the A and B genomes, respectively (You et al., 2004).

The question still remains in what extent the PIC value is related to the number of alleles at a given locus. In the present study, the PIC values per locus showed a significant, positive correlation with the number of alleles per locus for all accessions \((r = 0.82, P < 0.0001)\) and the European subset \((r = 0.71, P < 0.0001)\). The results are consistent with those of Huang et al. (2002) \((r = 0.73, P < 0.01)\) and Roussel et al. (2004) \((r = 0.69)\). Positive correlation between the PIC values per locus and the number of alleles per locus was also reported by Yu et al. (2003) and Jain et al. (2004) in rice \((r = 0.62, 0.72, \text{respectively})\), and by Vaz Patto et al. (2004) in maize \((r = 0.85)\). In contrast, Prasad et al. (2000) and Fu et al. (2005) reported that the PIC value per locus was not significantly associated with the number of detected alleles per locus. In fact of the different observation of correlation between the number of alleles per locus and PIC values, an objective evaluation of genetic diversity in germplasm collections should be considered, by both, the number of alleles per locus and their respective PIC values in combination as suggested by Hao et al. (2006).

How much loci (or alleles) are sufficient to precisely reveal the genetic diversity among accessions have been discussed by several authors (Tiang et al., 1994; Uptmoor et al., 2003). In the present study, it was clearly shown that little or no increase in CV of the PIC value was obtained with more 50 loci, corresponding
to ~400 alleles in the whole set data (69 accessions) and ~300 in the European subset (56 accessions), respectively. This result indicated that 52 SSR loci used in this study were enough to assess genetic variation. Similar results of Zhang et al. (2002) suggested that 350 - 400 alleles were required to objectively assess the relationship between wheat accessions.

Our cluster analysis based on two different methods led to similar groupings with only minor exceptions: e.g. two US varieties (IDO232 and Transec) as well as the variety B5769 (Switzerland) clustered in one of the subgroups of group 3 (UPGMA clustering), while these three varieties were not assigned to a separate group by structure analysis (Figure 3 and 4), but they were all assigned to group I with more than 90% shared ancestry each. The cv. Jordao was placed into group 2 by UPGMA clustering, however, as a result of structure analysis it was assigned to group I with 81.8% shared ancestry rather than to group III as would have been expected. Similar results showing minor deviation between UPGMA and structure analysis were reported by Lu et al. (2004) in rice where out of 145 cultivars, 139 were consistently grouped by both methods.

Concerning $G_{SDice}$, the present results showed a broad genetic variation in the whole set of genotypes ($G_{SDice} = 0.08 - 1.00$), with similar mean values for all accessions (0.31) compared to the European subset of spring wheats ($G_{SDice} = 0.35$). The maximum $G_{SDice}$ value of 1.00 was observed between cv. Amazonas and Avle, suggesting that these two genotypes are very closely related. Pedigree information was not extensive but there was not any hint that Amazonas originated from Portugal was equivalent to Avle from Sweden. AMOVA indicated that genetic variation within geographical groups had a major effect, contributing more than 80% of the total genetic variation. This is in agreement with the results of Roussel et al. (2005), where 92.3% of the total genetic variation in a set of 480 bread wheat varieties originating from 15 European areas could be explained by geographical origin. The higher proportion of genetic variation within groups found in the present study can be explained by breeding activities, i.e. the selection for germplasm adapted to local agro-ecological conditions as presumed by Roussel et al. (2005).

The relationship between the six European geographical gene pools discriminated in our study is comparable to Roussel et al. (2005). In agreement
with the latter authors, we found that clustering between six genetic groups was more related to pedigree-relatedness rather than geographical origin (Figure 7). For example, in the present study, in spite of geographical distance, wheats from CZ and E/P appeared in a common cluster (Figure 7), suggesting that there are genetically close to some extent. The relationship between the other four groups (A/CH, F/G/NL, N/S and UK) also reflected both the pedigree-relatedness as well as the geographical proximity. For example, the three German varieties Triso, Munk and Nandu and the Dutch cv. Baldus share a common parent (German cv. Kolibri). Correspondingly, these four varieties along with the German cv. Star (one of the parents of Munk) were assigned to group VI with more than 89% mean shared ancestry, whereas variety Nandu was assigned to this group as an admixture (50.4% shared ancestry; Figure 6). Accordingly, the pedigree relatedness between some varieties from the Netherlands and UK (group I) were also reflected in the results of structure analysis. For example, the two UK varieties Shiraz and Chablis have cv. Jerico (UK) as a common parent, which contributed to Bastion (UK), one of the parents of cv. Minaret (Netherlands) (Figure 7).

Focusing on European accessions, the overall genetic diversity of the six different geographical groups was $H_e = 0.65$, with varying values for the individual groups. The lowest $H_e$ value was detected for the CZ group ($H_e = 0.37$), which may be due to the small number of samples and just a few founder genotypes. For example, the varieties Linda, Saxana and Sandra share cv. Rena as a common parent, and Linda and Saxana were even selected from the same cross (Rena/ST802-74). This close relationship within the CZ group was elucidated by UPGMA cluster as well as by structure analysis. A similar explanation applies to the UK group, where a comparatively low diversity ($H_e = 0.42$) was detected due to the cultivars' close relatedness. For example, Jerico is a common parent of Shiraz and Chablis, and Axona is a parent of Shiraz and Cadenza; furthermore, Cadenza and Chablis share cv. Tonic as a common parent. On the contrary, the A/CH geographical group exhibited the highest genetic diversity ($H_e = 0.63$). This is reflected the fact that accessions from this origin were distributed to different groups by UPGMA as well as by structure analysis. The A/CH set of genotypes and the E/P wheats had a similarly high
genetic diversity ($H_o$ approx. 0.6) along with the highest number of alleles per locus. Correspondingly, Röder et al. (2002) reported that the highest diversity exists in Southern European wheats followed by Alps region. In agreement with this, Huang et al. (2002) observed a higher genetic variation in wheats from these countries as compared to wheat varieties from Western and Northern Europe such as Germany, Netherlands, Sweden and UK. Further on, Roussel et al. (2005) reported the highest number of alleles appeared in Spain and Portugal. In this context, it is worth mentioning that the correlation between the number of rare alleles per locus for each geographical area and $H_o$ value is rather close ($r = 0.89$, $P < 0.05$) whereas the correlation between the number of common alleles per geographical area and $H_o$ is weaker ($r = 0.83$, $P < 0.05$). Therefore, we assume that the higher genetic diversity observed in Southern European regions may be due to the presence of relatively more rare alleles, resulting from less stringent selection applied in these regions (Roussel et al. 2005). Studies of allele distribution in Nordic spring wheats throughout the 20th century, Christiansen et al. (2002) revealed that the loss of alleles only comprised of rare alleles in the new cultivars. This finding further confirmed our assumption that breeding practice affected the number of rare alleles.

The optimal strategies of breeding system require extensive knowledge of the breeding materials employed. The result of presented here will be useful to understand the current status of genetic diversity between accessions. Because most of SSRs markers applied in the present study were randomly selected from the whole wheat genome, exploring genetic variation for specific traits could not be expected. With advances in mapping of quantitative trait loci (QTL) for many agronomic important traits in wheat (Börner et al., 2002), it becomes possible to detect the allelic variation at these loci among accessions by using markers haplotypes. Haplotype information will allow the breeder to directly accumulate favourable alleles at multiple loci in a controlled manner leading to superior varieties (Peleman et al., 2003).

3.1.6 Acknowledgements

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3.2 Quantitative trait loci (QTL) for stem strength and related traits in a doubled haploid population of wheat (*Triticum aestivum* L.)

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3.2.1 Abstract

Scoring for lodging resistance is difficult under natural field conditions. The stem strength of wheat has been used as an index of lodging resistance. However, this is a complex trait comprised of two characters, i.e. stem mechanical elasticity and rigidity. Therefore it is closely associated with stem morphological and anatomical features. A study of the genetics of stem strength and related traits of basal stem internodes is very important for genetic improvement of lodging resistance in wheat. In this study, a doubled haploid (DH) population derived from anther culture of the cross CA9613/H1488 was used. Stem strength and related basal internode traits were measured at the milk stage. A molecular map of the DH population was constructed using 189 SSR markers, and quantitative trait loci (QTL) for each trait were analysed based on this
molecular linkage map. A total of six QTL for stem strength, culm wall thickness, pith diameter and stem diameter were identified: 1) Two QTL (QSs-3B) for stem strength were detected on chromosomes 3A and 3B, exhibiting 10.6 and 16.6% phenotypic variation, respectively. 2) Two QTL (QPd-1A and QPd-2D) associated with pith diameter were detected on chromosomes 1A and 2D, respectively, jointly explained about 30% of phenotypic variance. 3) As far as stem diameter and culm wall thickness were concerned, one QTL was detected on chromosomes 3B and 2D, respectively; Qsd-3B explained 8.7% of the phenotypic variance of stem diameter, whereas QCwt-2D explained 9.6% of the phenotypic variance of culm wall thickness. In addition, among the QTLs detected, two with pleiotropic effectes were observed. Correlated traits are usually associated with the pleiotropic effects of the same QTL(s) or linkage of different QTLs. But this was not true in some cases. The results of QTL mapping showed that stem strength can be improved by breeding for wider stems with a higher stem diameter/pith diameter ratio. This can be facilitated by using the markers linked to Qsd-3B and QCwt-2D. Combing stem strength, stem diameter and culm wall thickness may be used as a selection index for lodging resistance with marker-assisted selection (MAS) to improve lodging resistance in this population.

**Key word:** basal internode trait, doubled haploid; quantitative trait loci (QTL), stem strength; wheat (*Triticum aestivum* L.)

### 3.2.2 Introduction

Lodging is one of the major limiting factors to the production of cereals. It was estimated that lodging may cause up to 40% yield loss in wheat (Esson et al., 1993). The quality of the grain may deteriorate considerably due to increased grain moisture content and preharvest sprouting. Since the 1960s, with the introgression of dwarfing genes, lodging resistance has increased in wheat (Worland & Snape, 2001). It has been found that extreme dwarfism is also associated with several other undesirable characteristics, like decreased biomass, higher leaf density, shrunken kernels, premature senescence, and
increased susceptibility to diseases. However, lodging may still occur if stem strength is weak after dwarfing (Li, 1998). Consequently, efforts to improve stem strength should be an important focus in breeding wheat for lodging resistance. Since lodging can occur at the different stages of plant growth, scoring for lodging resistance is difficult under natural field conditions, and breeders have to establish methods of essentially assessing lodging resistance (Pu et al., 2000). Lodging usually occurs when the stems bend or break at the basal internode (Pinthus, 1973). Many studies have been conducted on the correlation between stem characters and lodging resistance, and no finding stem morphological and anatomical traits that can be used as indirect selection criteria in wheat. Some of these studies showed that lodging is negatively correlated with stem diameter and culm wall thickness, lodging resistance cultivars exhibited wider basal diameter and thicker culm wall than those susceptible to lodging (Muckherjee et al., 1967; Zuber et al., 1999). Such studies have also been reported for barley (Dunn & Briggs, 1989) and oats (Jellum, 1962).

However, Atkins (1938) and Pinthus (1967) found no significant correlation between stem diameter and lodging resistance in wheat. Kelbert et al., (2004) conducted a study to determine the association between culm anatomy and lodging using 13 spring wheat cultivars differing in lodging susceptibility, and slaos did not find stem diameter to be a significant character related to lodging resistance. Rather, three lodging resistant cultivars had shorter, wider basal internodes and thicker culm walls. Moreover, Luthra et al. (1981) determined that F₁ hybrids from a diallel cross involving seven wheat cultivars differed significantly from the parents in characteristics related to lodging including plant height, stem strength, stem diameter and length of second internode. Wang et al. (1998) reported that using systematic cluster analysis based on the stem strength differences at different growth stages, 15 high-yielding wheat varieties were divided into four lodging resistant types, and highly significant differences of stem strength between wheat varieties at different stages were found. Berry et al. (2000) suggested that combining stem strength with other desirable characteristics remains a major goal in breeding for lodging resistance.

Stem strength seems to be a complex trait including mechanical elasticity and
rigidity of the stem and is therefore closely associated with stem morphological and anatomical traits (Wang & Li, 1996). Related studies by some researchers showed that stem strength was correlated with stem diameter of the basal internodes \((r = 0.80, \text{Shevchuk et al., 1981}; r = 0.87, \text{Min, 2001})\). In another study, Xiao et al. (2002) found that the diameter of basal internodes was correlated with stem strength from the milk to maturity stage \((r = 0.379, 0.498\) and 0.461), while the diameter of the upper internodes was not positively related to stem strength. Based on classical quantitative genetic methods, experiments have been performed to determine the genetics of stem strength and related traits.

Both stem strength and stem diameter were normally distributed in the F\(_2\) population, with significantly transgressive segregation as usually observed for quantitative traits (Kohli et al., 1970; Xiao et al., 2002). Li (1998) reported that stem strength, stem diameter and pith diameter were controlled by both additive and non-additive gene effects.

The development and utilization of DNA molecular markers and genome mapping techniques have facilitated the identification of QTL for complex traits (Lander & Bostein, 1989; Tanksley, 1993). With the QTL mapping approach, it is feasible to analyse the genetic basis of the relationship between traits (Lin et al., 1996; Kato et al., 2000; Ishimaru et al., 2001). This will be useful for genetic improvement of lodging resistance in wheat. In this study, we attempt to identify QTL affecting stem strength, stem diameter, culm wall thickness and pith diameter of basal internodes in a DH population, and to explore their utilization in wheat lodging resistance breeding by means of marker-assisted selection (MAS).

### 3.2.3 Materials and methods

#### 3.2.3.1 Plant materials

A doubled haploid (DH) population consisting of 113 lines established in our laboratory were used. This population was developed through anther culture of F\(_1\) hybrid derived from the cross between the winter wheat cultivars ‘CA9613’
with a tenuous stem as female parent and the winter wheat cultivar 'H1488' with a strong stem as male parent. The 113 DH lines and their parents were planted during the periods 2000-2001 and 2001-2002 at the experimental station of Institute of Crop science, Chinese Academy of Agricultural Sciences, Beijing. The field experiment was conducted in a randomized block design with three replications. Each experimental unit contained one row (2m) with 25 cm row space. One hundred seeds were sown in each row.

3.2.3.2 Measurement of stem strength and related basal internode traits

The prostrate tester (DIK-7400, Daiki Rika Kogyo Co. Ltd., Tokyo, Japan) was used to measure the stem strength of plants in the study (Figure 10). The instrument functions on the basis of the principle of action and reaction. Stem strength was measured at the milk stage according to the method of Xiao et al. (2002). The internodes were numbered from top to bottom. The prostrate tester was set perpendicularly at the middle of the second internode of the plant. The stem strength was measured when the plant was pushed to an angle of 45 from the vertical and it was estimated using the following formula: Stem strength (g/stem) = test reading ÷ 40 × 1000 ÷ number of stems. Five stems were measured in each experimental unit (plot). Stem diameter (mm) and culm wall thickness (mm) were measured at the center of the fifth internode using a sliding caliper. Pith diameter was calculated according to the equation: stem diameter = culm wall thickness + pith diameter.

Figure 10. Measurement of stem strength using the Prostrare tester. The prostrare tester was set perpendicularly at the middle of the 2nd internode on the aerial parts of plants (a). The stem strength was measured when plant was bent to 45° (b). (Sketch cited from the study of Kashiwagi et al. (2004) with thanks, some changes have been made with respect to his original study.).
Table 8 Descriptive statistics of stem strength, stem diameter, pith diameter and culm wall thickness of basal internode on the DH population and their parents

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>CA9613</th>
<th>H1488</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem strength</td>
<td>8.11 ± 5.54</td>
<td>0.1427-27.46</td>
<td>7.63</td>
<td>17.79</td>
</tr>
<tr>
<td>Stem diameter</td>
<td>3.53 ± 0.35</td>
<td>2.75-4.38</td>
<td>3.42</td>
<td>3.53</td>
</tr>
<tr>
<td>Pith diameter</td>
<td>1.46 ± 0.46</td>
<td>0.63-3.00</td>
<td>1.29</td>
<td>1.77</td>
</tr>
<tr>
<td>Culm wall thickness</td>
<td>2.08 ± 0.40</td>
<td>0.75-2.87</td>
<td>2.13</td>
<td>1.76</td>
</tr>
</tbody>
</table>

3.2.3.3 SSR analysis

The microsatellite markers developed by Röder et al. (1998) and by the Wheat Microsatellite Consortium were used in this study. PCR reactions were performed in a programmable thermal controller (PTC-100, MJ Research Inc., Watertown, MA, USA) in a total volume of 20µl containing 1 × buffer (Promega), 1.8 mmolL⁻¹ MgCl₂, 200 mmolL⁻¹ dNTPs, and 250 nmolL⁻¹ of each primer, 1U of Taq-polymerase, 100ng of genomic DNA as template. After an initial denaturing step for 5 min at 95°C, 35 cycles were performed for 40s at 94°C, 30s at either 50°C, 55°C, or 60°C (depending on the primer pair), 45s at 72°C, followed by a final extension step of 10 min at 72°C. Amplification products were separated on 5% (w/v) denaturing polyacrylamide gels and were detected by silver staining according to the protocol of Bassam et al. (1991).

3.2.3.4 Molecular map construction

Genetic linkage maps were constructed by MAPMAKER/Exp version 3.0b (Land et al., 1987). A threshold log likelihood ratio (LOD) of 3.0 was used to arrange markers into linkage groups. The Kosambi (1994) mapping function was applied to transform recombination frequencies into centiMorgans. Linkage groups were assigned to chromosomes via comparison to reference maps using microsatellite loci (Röder et al., 1998).

3.2.3.5 Statistical analysis

Statistical analysis of traits was performed using the SAS statistics package (SAS Institute, Raleigh, NC, USA). Normality of each trait measured was
verified suing the “PROC UNIVERIATE” procedure was performed using “PROC CORR” procedure. QTL detection was performed by Composite interval mapping (CIM) (Zeng, 1994) using the program QTL Cartographer 1.3 (Basten et al., 1997). A forward and backward stepwise regression was performed to choose factors before QTL detection by CIM. Ten cofactors with the highest F value were taken into account. A window size of 10 cM around the test interval was chosen for all analyses. Permutation tests were performed to estimate appropriate significant thresholds for CIM. After 1000 permutations, LOD thresholds of 2.5 were chosen for CIM. For each QTL, the position, the additive effects(s), and the percentage of phenotypic variation explained were estimated.

3.2.4 Results

3.2.4.1 Variation in stem strength and correlation between stem strength and related basal internode traits

Parental performance and segregation for stem strength, stem diameter, pith diameter and culm wall thickness of basal internode are shown in Table 8 and Figure 11. The measured values of stem strength, stem diameter and pith diameter were greater for ‘H1488’ than for ‘CA9613’, whereas the measured value of culm wall thickness was greater for ‘CA9613’ than for ‘H1488’. Significant differences were found in stem strength and pith diameter between two parents (P <0.05), whereas differences in stem strength and culm wall thickness were not significant. All the four traits were normally distributed with transgressive segregation in the DH population. Correlation coefficients between stem strength, stem diameter, pith diameter and culm wall thickness in the DH population are shown in Table 9. Stem strength was significantly correlated to stem diameter (r = 0.143, P <0.05) and culm wall thickness (r = 0.196, P <0.05), whereas there was a negative relationship between stem strength and pith diameter (r = -0.064). Coefficients between stem diameter and pith diameter (r = 0.529, P <0.001) and between stem diameter and culm wall thickness (r = 0.259, P <0.01) were both positively significant, whereas there was negatively significant correlation between pith diameter and culm wall...
thickness ($r = -0.682, P < 0.001$).

### 3.2.4.2 Molecular map

Among 771 microsatellite markers, 200 were identified as polymorphic between ‘CA9613’ and ‘H1488’, and these markers were used to assess the marker genotype of the DH lines to construct a molecular map. The map comprised 189 markers on 25 linkage groups with two or more markers each. The total length of the present map is 2308.3cM. The linkage groups are distributed throughout the wheat genome but chromosomes 1D, 4A, 4D, 5A, 6A and 6D were underrepresented. Among all 189 markers, segregation significantly deviating from the expected 1:1 ratio was determined by Chi-square tests. A total of 59 markers (31.38%) showed distorted segregation at $P < 0.05$; and 36 markers (19.15%) showed distorted segregation at $P < 0.01$. Most of these markers map to chromosomes 1D, 2A, 2D, 3B, 6A and 7A.

**Figure 11** Distribution of stem strength, stem diameter, pith diameter and culm wall thickness of basal internode in the DH population.
Table 9: Correlation coefficients between stem strength, stem diameter, pith diameter and culm wall thickness of basal internode in the DH population

<table>
<thead>
<tr>
<th>Trait</th>
<th>Stem strength</th>
<th>Pith diameter</th>
<th>Stem diameter</th>
<th>Culm wall thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem strength</td>
<td>1.000</td>
<td>-0.064</td>
<td>0.143*</td>
<td>0.196*</td>
</tr>
<tr>
<td>Pith diameter</td>
<td>-0.064</td>
<td>1.000</td>
<td>0.529***</td>
<td>-0.682***</td>
</tr>
<tr>
<td>Stem diameter</td>
<td>0.143*</td>
<td>0.529***</td>
<td>1.000</td>
<td>0.259**</td>
</tr>
<tr>
<td>Culm wall thickness</td>
<td>0.196*</td>
<td>-0.682***</td>
<td>0.259**</td>
<td>1.000</td>
</tr>
</tbody>
</table>

* and ** indicate 0.05, 0.01 and 0.001 significant level, respectively.

Table 10: QTLs associated with stem strength, stem diameter, pith diameter and culm wall thickness of basal internode detected by CIM in the DH population derived from CA9613 and H1488

<table>
<thead>
<tr>
<th>Traits</th>
<th>QTLs</th>
<th>Chr.</th>
<th>Marker Interval</th>
<th>LOD*</th>
<th>A²</th>
<th>R² (%)</th>
<th>Positive Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem strength</td>
<td>QSs-3A</td>
<td>3A</td>
<td>xwmc527 - xwmc21</td>
<td>3.19</td>
<td>2.37</td>
<td>10.61</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>QSs-3B</td>
<td>3B</td>
<td>xgwm108 - xwmc291</td>
<td>4.11</td>
<td>3.12</td>
<td>16.60</td>
<td>H</td>
</tr>
<tr>
<td>Stem diameter</td>
<td>QSD-3B</td>
<td>3B</td>
<td>xgwm108 - xwmc291</td>
<td>2.75</td>
<td>0.11</td>
<td>8.75</td>
<td>H</td>
</tr>
<tr>
<td>Pith diameter</td>
<td>QPD-1A</td>
<td>1A</td>
<td>xgwm135 - xwmc84</td>
<td>2.81</td>
<td>0.16</td>
<td>10.72</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>QPD-2D</td>
<td>2D</td>
<td>xgwm311 - xgwm301</td>
<td>5.29</td>
<td>0.21</td>
<td>18.70</td>
<td>H</td>
</tr>
<tr>
<td>Culm wall thickness</td>
<td>QCWT-2D</td>
<td>2D</td>
<td>xgwm311 - xwmc301</td>
<td>2.93</td>
<td>-0.14</td>
<td>9.63</td>
<td>C</td>
</tr>
</tbody>
</table>

*a Log10-likelihood value.
b Additive effect: its positive value indicates the genotype from parent ‘H1488’ toward increasing the phenotype value.
c Variance explained by each QTL.
d Positive allele was derived from ‘H1488 (H)’ or ‘CA9613 (C)’.

3.2.4.3 QTL detection

QTL controlling stem strength, stem diameter, pith diameter and culm wall thickness of basal internode were detected in the DH population CA9613/H1488 (Table 8, Figure 12). Two putative QTLs (QSs-3A and QSs-3B) for stem strength were detected on chromosomes 3A and 3B which individually explain 10.6 and 16.1% of phenotypic variance, respectively; and the positive alleles of both derived from parent ‘H1488’. Two QTLs (QPD-1A and QPD-2D) associated with pith diameter were mapped to chromosomes 1A and 2D; and these QTL explained 10.7 and 18.7% of phenotypic variation, respectively; the positive alleles of both also derived from the parent ‘H1488’. One QTL (QSs-3B) affecting stem diameter was detected on chromosome 3B, and it explained
8.7% of the phenotypic variance. For culm wall thickness, one QTL (QCwt-2D) was detected on chromosome 2D, explained 9.6% of phenotypic variance of stem thickness. The positive allele of QSd-3B derived from 'H1488', which the positive allele of QCwt-2D derived from 'CA9613'.

![Figure 12](image_url) The most likely location of QTLs for stem strength and related traits in a wheat DH population derived from a cross between 'CA9613' and 'H1488'.

### 3.2.4.4 Pleiotropic effects

Among the QTL detected, two loci with pleiotropic effects were observed. One locus in the interval xgwm108-xwmc291 on Chromosome 3B simultaneously influenced stem strength and stem diameter; the other locus in the interval xgwm311-xgwm301 on chromosome 2D is simultaneously associated with pith diameter and stem thickness, but positive alleles were derived from both parents.

### 3.2.5 Discussion

Lodging is a complex trait affected by many morphological and anatomical traits. Therefore selection based on phenotype for lodging-resistant genotypes is very difficult. Marker-assisted selection could therefore become an important tool in breeding for this trait (Keller et al., 1999). Lodging resistance determined by
stem strength in wheat has been reported earlier (Mulder, 1954; Malkani & Vaidga, 1956; Wang & Li, 1995). Stem strength as an index of lodging resistance has been used in rice (Terashima et al., 1992) and in wheat (Xiao et al., 2002). However, few studies have reported on QTL analysis of these traits in wheat.

In the present study, stem strength, stem diameter, pith diameter and culm wall thickness in the DH population showed normal distributions, indicative of quantitative traits. This is in conformity with the results previously reported by Kohli et al. (1970), Li (1998) and Xiao et al. (2002). In total six QTL were detected for stem strength and related stem traits; two QTL (QSs-3A and QSs-3B) associated with stem strength were detected on chromosomes 3A and 3B, respectively. The positive alleles for both QTL were derived from ‘H1488’, a cultivar with a strong stem. Also, the chromosome region QSs-3B was shown to exhibit pleiotropic effect(s). The locus flanked by markers xgwm108 and xwmc291 on chromosome 3B simultaneously affected stem strength and stem diameter. Again, the positive allele of this QTL was from ‘H1488’ with an additive effect for an increased stem strength and stem diameter. Based on a cytogenetic study for location of genes associated with lodging on this chromosome, Al-Qaudhy et al. (1988) found that chromosomes 3A and 3B of wheat had major effects in increasing stem strength. The in the present study, one QTL associated with stem-breaking strength was additionally detected on chromosomes 3A (data not shown). Together with the results of Al-Qaudhy et al. (1988), it is presumed that there may be QTLs on both chromosomes 3A and 3B associated with stem strength.

Correlated traits are often associated with pleiotropic effects of the same QTL or linkage of different QTL as reported for heading data and plant height in barley (Qi et al., 1998), oats (Holland et al., 1997) and for grain number and grain weight in rice (Xing et al., 2002). In our study, chromosomes regions of QPd-2D and QCwt-2D are different from that of QSs-3B and Qsd-3B. The positive effect for QPd-2D was contributed by ‘H1488’, whereas the positive effect for QCwt-2D was derived from ‘CA9613’. The results were consisted with the results of the correlation analysis for these traits in this population, where significant negative correlations were observed between pith diameter and culm wall.
thickness ($r = -0.682, P < 0.001$). Similar results were reported for rice by Yamagishi et al. (2002). However, they were not always correct. For example, stem diameter was highly co-associated with pith diameter compared to other traits, which were significantly and positively related with each other, although no identical QTL was detected for these two traits. Bao et al. (2002) reported that no common QTL was identified for gelatinization temperature and viscosity breakdown associated with rice grain quality, while a highly significant correlation between these two traits was found.

Our results of QTL mapping show that stem strength can be improved by breeding for stem thickness and higher stem diameter/pith diameter ratio. This can be facilitated by using markers linked to $QSd-3B$ and $QCwt-2D$. Combined stem strength, stem diameter and culm wall thickness may be used in combination as a selection index to be used in MAS for improving lodging resistance in this population. However, further studies are needed to verify: 1) the stability of QTLs detected in this population to different population(s) including DH and RIL populations derived from the same cross, and also in diverse environments; and 2) the chromosome regions with pleiotropic effects identified in this study. Fine mapping will be necessary to determine the pleiotropic effects of a single QTL or a tight linkage of two QTL in the same region. Based on corresponding results, a strategy for application of MAS in breeding for lodging resistance in wheat may be developed.

It needs to be stressed that stem strength is not only associated with morphological and anatomical traits of the stem, but also associated with several physiological plant trait. According to Huang (1988) and Li (1998), the soluble carbohydrate content in basal internodes of the stem was significantly correlated with lodging resistance, and the lignin content of basal internodes of strong stems was higher than that of weak stems. Similar results have been found in rice. For example, Taylor et al. (1999) found that lignin content is related to stem rigidity, and higher contents accumulated carbohydrates in rice stem contributed greatly to lodging resistance (Sato, 1957; Yang et al., 2001). In addition, Matsuzaki et al. (1972) showed that higher accumulation of starch contributed to high bending strength.

In the future, we are planning to map QTL affecting physiological factors
responsible for or involved in stem strength, and to elucidate the physiological functions of QTL associated with stem strength and related traits in our DH population. This may contribute to a better understanding of how to improve stem strength by breeding.

3.2.6 Acknowledgements

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3.2.7 References

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4 DISCUSSION

4.1 Genetic variation in existing gene pools of spring wheat (*T. aestivum*)

It is a basic requirement for the genetic improvement of crop plants such as bread wheat to characterize the range and structure of genetic variation among elite lines and cultivars in existing gene pools. Both, the magnitude of variation and heritability directly determine the result and success of combination breeding.

The patterns of variation of microsatellite (SSR) markers presented here provide a comprehensive characterization concerning the level and distribution of genetic diversity among spring wheat accessions originating from different geographic regions of the world. This work is also expected to make a useful contribution to the discussion related to the efficient conservation and utilization of wheat germplasm resources.

For example, in the present study evidence for unequal diversity and differentiation among wheat accessions was found. The results of analysis of molecular variance (AMOVA) indicate that genetic variation within geographical groups has a major effect, contributing more than 80% of the total genetic variation. This finding is in agreement with a previous report by Roussel et al. (2005) on a set of 480 wheat varieties originating from 15 European geographical regions where 92.3% of the total genetic variation could be explained by geographical origin. In particular, it was found in the present study that a higher genetic diversity along with a higher allelic richness appeared in accessions belonging to the European geographical groups Austria/Switzerland and Spain/Portugal. Similar results showing that these two regions exhibit a high genetic diversity and allelic richness have been presented in the studies of Röder et al. (2002), Huang et al. (2002) and Roussel et al. (2005). The higher genetic diversity observed in Southern European regions may be the results of both, i) adaptation of the initial germplasm to different environmental conditions, and ii) specific breeding practices (Roussel et al., 2005).

Further on, in the present study, a high number of rare alleles has been
observed among wheats from these regions, especially Spain/Portugal, where 27.5% of all alleles detected belong to rare alleles. This may be the result of the combined effects of the two major factors mentioned above, where rare alleles may be associated with favourable traits adapted to local agro-ecological conditions. Obviously, such accessions should receive a high priority in germplasm conservation.

On the whole, the different levels of diversity observed in European geographical groups suggest that more attention needs to be paid to the maintenance of the existing germplasms and to increase the exchange of genetic resources to exploit the whole range of allelic variation.

4.2 Exploration of desirable alleles in genetic resources

Since the SSR markers used in this study are presumably neutral (or non-functional) and their polymorphism may not contribute directly to the variation in traits of interest, exploring genetic variation for specific agronomic or useful traits could not be expected. However, with the rapid development of molecular marker techniques, several approaches have been proposed or applied for understanding the allelic diversity in breeding populations.

One of these approaches is based on the genetic mapping of quantitative trait loci (QTL). Many QTL for traits of agronomic relevance such as grain yield and related characteristics have been identified in wheat so far (Börner et al., 2002; McCartney et al., 2005; Huang et al., 2006). This may enable the determination of the allelic variation at loci of agronomic importance among distinct accessions using tightly linked markers for these loci.

Moreover, a great number of ESTs (expressed sequence tags) have recently become available for bread wheat. Functional molecular markers such as EST-SSRs and single nucleotide polymorphisms (SNPs) developed from ESTs provide very powerful tools to reveal the functional polymorphism(s) at specific gene loci (Rafalski, 2002). Since such markers represent part(s) of the expressed gene sequence they are completely linked to the functional allele(s) encoding the desired trait expression. Therefore, they have a clear advantage over non-functional markers such as SSRs, which are generated from
anonymous regions of the genome (Varshney et al., 2005). With the functional markers-approach, the allelic variation at specific genetic loci can be directly identified in genetic resources, for example of bread-making wheat. Recently, trait-allele association studies based on linkage disequilibrium (LD), defined as the non-random association of alleles, have been paid more attention in crop plants (Buckler et al., 2002). Only polymorphisms with extremely tight linkage to a locus are likely to be significantly associated with the trait in natural populations or germplasm collections (Remington et al., 2001). Therefore, it is probably possible with this approach to correlate the genetic diversity with phenotypic variation and allow the identification of the actual genes (alleles) responsible for QTLs (Garris et al., 2003).

Since the resolution of association studies depends on the genomic structure of LD, understanding the extent of LD is critical for the success of an association study (Reif et al., 2005). In the present work, using a set of 52 SSR markers, we have attempted to test LD between pairs of loci within the set of wheat accessions used. However, since small sets of SSR markers applied are not sufficient to conduct a genome scan, no evidence could be found for significant LD. Further studies using a larger number of SSR markers are necessary for this purpose.

4.3 Quantification of lodging resistance as a major stability trait of wheat

An objective estimation of lodging resistance is critical for breeding research. Several methods are available to evaluate the severity and effect lodging. The most frequently used approach is visual ranking (Verma et al., 2005; Huang et al., 2006; McCartney et al., 2005). However, due to the fact that the degree of lodging is subject to the environmental conditions, this scoring method of lodging can result in a biased estimation. Another common method is hand scoring of culm stiffness (Keller et al., 1999). This method is simple, however, the problem is its repeatability, since different breeders may give different scores for the same materials, especially in large populations.

Other procedures are based on stem strength, i.e. the mechanical elasticity and rigidity of the stem which is considered to be closely related to lodging
resistance (Wang et al., 1996; Pu et al., 2001). Several methods have been proposed and applied for evaluating lodging resistance: For example, measuring the stem-breaking strength (Wang et al., 1995) or testing the pushing resistance of the stem (Xiao et al., 2001). As compared to the measurement of the stem-breaking strength, testing the pushing resistance of the stem seems to be a more suitable approach for estimating lodging resistance. This method can be performed by a special instrument and lodging scores can be automatically recorded. Furthermore, this approach enables to quickly, repeatedly and directly test a large number of wheat breeding materials without any damage (Xiao et al., 2002).

Several instruments, which generally function on the basis of the principle of action and reaction, have been developed and are available to measure the stem strength. Such instruments have been used in several studies (Kashiwagi & Ishimaru, 2004; Zhu et al., 2004; Wang et al., 2006). For example, using a prostrate tester, Xiao et al. (2002) successfully measured the stem strength of 661 varieties and of 1,183 single plants from an F2 population of bread wheat. A large variation of stem strength ranging from 0 to 68 g/stem was observed, indicating that this method was very effective for assessing the phenotypic variation of lodging resistance. Using the prostrate tester and the same method as in the study mentioned above, a significant difference of stem strength between two parental wheat varieties (CA9613 and H1488) was detected in the present work, and a variation of stem strength from 0.14 to 27.46 g/stem was found amongst 113 DH lines derived from a cross of these two parents.

Regarding the method used, it should be mentioned that the test instrument was set perpendicularly at the middle of the 2nd internode (counted from the top) of the plant. Thus, the stem strength value measured reflects the combined stem strength of the internodes under the 2nd internode and the effect of plant height could thereby be eliminated to some extent (Xiao et al., 2002).

4.4 QTL mapping of stem strength and perspectives of marker-assisted selection for lodging resistance

QTL for lodging resistance of bread wheat determined by visual ranking have
been reported in several studies. These QTL mainly involved as many as 11 of the 21 wheat chromosomes, i.e. 1B, 1D, 2B, 2D, 4A, 4B, 4D, 5A, 6A, 6D and 7D (Börner et al., 2002; Verma et al., 2005; Marza et al., 2006; McCartney et al., 2005; Huang et al., 2006). However, in the present work no significant QTL for stem strength as an indicator of lodging resistance were found on the chromosomes mentioned above. Instead, two QTL associated with stem strength were mapped on chromosomes 3A and 3B, respectively, and were putatively named QSs-3A and QSs-3B. The different results can be explained by the fact that visual ranking was used for scoring lodging resistance in the studies above, and stem strength was measured as an indirect criterion for lodging resistance in the present study. As mentioned above, lodging is not only determined by genetic but also by environmental factors. Therefore, further studies are necessary to identify the main genes involved in and responsible for resistance of wheat against lodging as a major trait determining standability and yield stability of the cereal crop. For example, using visual ranking of lodging resistance and to conduct QTL mapping for this trait in our DH population in order to compare the results of QTL mapping of the two different scoring methods would be helpful to further elucidate the relationship between stem strength and lodging resistance.

As discussed above, lodging is easily affected by the respective environmental conditions. Thus, the identification of stable QTL across different environments is important for their further application in markers-assisted selection (MAS) programmes. However, since the QTL analysis was conducted based on the average data across two environments, the environmental effects could not be determined in the present work. Therefore, the expression of QTLs in different environments needs to be analysed in a further study.

In addition, two loci with pleiotropic effects were detected in the present study. One of the pleiotropic loci was identified in the marker interval xgwm108–xmw2c91 on wheat chromosome 3B, simultaneously affecting stem strength and stem diameter. The positive allele of this QTL was derived from the parental line ‘H1488’ and has an additive effect regarding increased stem strength and stem diameter. Another locus in the marker interval xgwm311–xgwm301 on chromosome 2D has a pleiotropic effect on the pith diameter and the culm wall
thickness; the positive allele of QCwt-2D (affecting culm wall thickness) was derived from parental line ‘H1488’, whereas the positive allele of QPd-2D (pith diameter) originates from the parental line ‘CA9613’. Based on these two loci it may be concluded that increasing the stem diameter and culm wall thickness and decreasing pith diameter may result in an increased stem strength.

Further on, the stem strength can be improved by selecting for wider stems and a higher stem diameter/pith diameter ratio. This may be facilitated by combining the markers linked to QSs-3A, QSd-3B (QPd-3B) and QCwt-2D (QPd-2D).

Moreover, it can be selected for the markers closely linked to the stem strength QTL to analyse the allelic variation in our collection of elite wheat germplasm by marker haplotyping. Further, by trait-allele association studies combing the bread wheat haplotypes with phenotypic values favourable alleles associated with stem strength can be determined. This will provide useful tools for the selection of lodging resistant elite lines and wheat cultivars for bread-making purposes by MAS programmes.

4.5 References


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5 SUMMARY

Effective crop improvement depends on the existence of genetic diversity. Therefore, it seems necessary to understand the levels and distribution of genetic diversity in existing crop gene pools, as a basis for developing strategies of resource management and exploitation.

In the first part of this study, fifty-two SSR markers were used to characterize the genetic diversity among 69 varieties of spring bread wheat (Triticum aestivum L.). The number of alleles per locus ranged from 2 to 14 with an average of 7.81. The largest number of alleles per locus occurred in the B genome (8.65) in comparison to the A (8.43) and D genomes (5.93). In total, 406 alleles were detected in the whole set of 69 accessions, of which 182 (44.8%) occurred at a frequency of <5% and are considered as rare alleles varying from 0 (xgwm129, wmc307, wmc245, wmc233 and barc196) to 9 (wmc326). The PIC values of the 52 SSR loci ranged from 0.24 to 0.90 with an average of 0.68. The highest PIC value for all accessions was identified for the B genome (0.71) compared to the A (0.68) and the D genomes (0.63).

Analysis of molecular variance (AMOVA) showed that 80.6% of the total variation could be explained by the variance within the geographical groups. Genetic distance-based method (standard UPGMA clustering) and a model-based method (structure analysis) were used for cluster analysis. Distance-based UPGMA cluster analysis divided all 69 accessions into five main groups, while by the model-based structure analysis the 69 accessions were assigned to nine genetically distinct groups, except 22 accessions identified as admixtures having 37.5-78.3% shared ancestry with one of the major groups. In comparison to UPGMA clustering, structure analysis led to analogous results.

The mean genetic diversity between European accessions is estimated at 0.65 ($H_e$) compared to $H_e = 0.68$ for all accessions. Regarding each geographical group, a higher diversity was found within A/CH ($H_e = 0.63$) and E/P ($H_e = 0.62$), whereas a lower diversity was observed in the pools F/D/NL ($H_e = 0.55$) and N/S ($H_e = 0.51$), and especially within UK ($H_e = 0.42$) and CZ ($H_e = 0.37$). The number of alleles per locus was in the same order, starting with the highest
values within A/CH (2.92), E/P (2.88) and F/D/NL (2.56), followed by N/S (2.38), UK (2.01) and CZ (1.84).

Based on the results of the present study, we suggest that more attention needs to be paid to maintain the already existing germplasm collections and increase the exchange of germplasm resources for the exploitation of the whole range of allelic variation.

The exploitation of existing germplasm is particularly relevant for further improvements of yield performance and yield stability. As one of the major limiting factors for yield stability and the production lodging regularly causes severe yield damages in cereals like wheat (*T. aestivum*). Exact scoring of lodging resistance is difficult under natural field conditions. The stem strength of wheat has been used as an index of lodging resistance. Efforts to improve stem strength should be an important focus in breeding wheat for lodging resistance.

In this study, a doubled haploid (DH) population derived from anther culture of the cross CA9613 x H1488 was used. A molecular map of the DH population was constructed based on microsatellite (SSR) markers. Stem strength and related basal internode traits were measured at the milky stage of grain development. Quantitative trait loci (QTL) for stem strength and related traits were analyzed based on this molecular linkage map. Two putative QTLs (QSs-3A and QSs-3B) for stem strength were detected on chromosomes 3A and 3B, which individually explain 10.6 and 16.1% of phenotypic variance, respectively, and the positive alleles of both derived from parent ‘H1488’. Two QTLs (QPd-1A and QPd-2D) associated with pith diameter were mapped to chromosomes 1A and 2D; and these QTL explained 10.7 and 18.7% of phenotypic variation, respectively; the positive alleles of both also descended from the parent ‘H1488’. One QTL (QSs-3B) affecting stem diameter was detected on chromosome 3B, and it explained 8.7% of the phenotypic variance. For culm wall thickness, one QTL (QCwt-2D) was detected on chromosome 2D, explaining 9.6% of phenotypic variance of stem thickness. The positive allele of QSd-3B derived from ‘H1488’, whereas the positive allele of QCwt-2D descended from ‘CA9613’. Combining the results of the QTL mapping for strength and related traits, it is assumed that increasing the stem diameter and culm wall thickness and
decreasing pith diameter can result in an enhanced stem strength. Further on, the stem strength can be improved by selecting for wider stems and a higher stem diameter/pith diameter ratio of the culm. This can be facilitated by a combined selection for the markers linked to QSs-3A, QSd-3B (QSd-3B) and QCwt-2D (QPd-2D) in marker-assisted breeding in this as well as other wheat populations.
Eine erfolgversprechende Verbesserung von Kulturpflanzen setzt das Vorhandensein und die Nutzbarkeit genetischer Diversität voraus. Eine Kenntnis über den Grad und die Verteilung genetischer Diversität stellt daher eine wichtige Grundlage für die Entwicklung geeigneter Strategien zur optimalen Nutzung der verfügbaren genetischen Ressourcen dar.


Fokussiert auf das europäische Subset von 56 Varietäten, wurden 320 polymorphe Allele detektiert, worunter sich 100 seltene Allele befinden. Insgesamt zeigte sich im europäischen Subset eine geringere Anzahl von Allelen pro Locus im Vergleich zur Gesamtpopulation aller Akzessionen. Im Hinblick auf die PIC-Werte ergaben sich vergleichbare Werte für das A- und D-Genom, während das B-Genom wiederum den höchsten PIC-Wert aufwies. Zwischen den PIC-Werten und der Anzahl der Allele wurde für alle 69 Genotypen sowie das europäische Subset allein eine signifikante Korrelation festgestellt (*r* = 0,82 bzw. 0,71; *P* <0,001).

Um den Einfluß der Stichprobengröße auf die Genauigkeit der Schätzung der

Um die genetische Ähnlichkeit auf molekularer Ebene zwischen den 69 Sorten näher zu charakterisieren, wurde die paarweise genetische Ähnlichkeit nach DICE (GS\textsubscript{DICE}) ermittelt und im weiteren eine UPGMA-Clusteranalyse durchgeführt. Parallel dazu wurde anhand der SSR-Fingerprints eine Modell-basierte Struktur-Analyse durchgeführt. Die genetische Ähnlichkeit zeigte eine große Variation von GS\textsubscript{DICE} = 0,08 bis 1,00 mit einer durchschnittlichen genetischen Ähnlichkeit von GS\textsubscript{DICE} = 0,31 aller Varietäten und GS\textsubscript{DICE} = 0,35 im europäischen Subset. Die UPGMA-Clusteranalyse ergab eine Differenzierung der 69 Akzessionen in fünf Hauptgruppen, wobei nahezu alle europäischen Sorten in zwei größeren Gruppen zusammengefasst sind.


Die in die Untersuchung einbezogenen 69 Sommerweichweizen-Genotypen können 10 geographischen Herkünften zugeordnet werden, wobei die europäischen Akzessionen (56 Sorten) sechs geographische Regionen repräsentieren: Österreich/Schweiz (A/CH), Tschechien (CZ), Portugal/Spanien
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(E/P), Frankreich/Deutschland/Niederlande (F/D/NL), Norwegen/Schweden (N/S) und Großbritannien (UK). Eine AMOVA wurde auf beiden hierarchischen Ebenen (Gesamtset und europäisches Subset) zur Bestimmung des Anteils an der Gesamtvarianz, welcher durch die Variabilität zwischen bzw. innerhalb der 10 geographischen Gruppen bzw. zwischen und innerhalb der europäischen Gruppen erklärt wird, durchgeführt. Auf beiden hierarchischen Ebenen war die Variabilität hoch signifikant, wobei der Anteil der Variabilität innerhalb der Gruppen mit 80,6% bzw. 84,4% an der Gesamtvarianz aller Genotypgruppen beziehungsweise der europäischen Sortengruppen bestimmend ist.

Die genetische Variation innerhalb des europäischen Materials wurde im Hinblick auf die genetische Distanz und Divergenz näher charakterisiert. Basierend auf Nei's stichprobenunabhängiger genetischer Distanz konnten nach UPGMA-Clusteranalyse zwei Cluster deutlich differenziert wurden: (1) CZ und E/P sowie (2) A/CH, F/D/NL, N/S und UK.

Der $F_{st}$-Index zur Beschreibung der genetischen Differenzierung der europäischen geographischen Gruppen wurde basierend auf allen 52 SSR-Loci bestimmt. Die paarweisen $F_{st}$-Werte zeigen eine signifikante Differenzierung der Gruppe F/G/NL zu den Gruppen A/CH, E/P und UK sowie der Gruppe A/CH und E/P von der Gruppe UK.

Die mittlere genetische Diversität zwischen den europäischen Gruppen ist mit $H_e = 0,65$ geringfügig niedriger als in der Gesamtpopulation ($H_e = 0,68$). Bei einer differenzierten Betrachtung der jeweiligen europäischen Gruppen wurde eine größere Diversität innerhalb A/CH/ ($H_e = 0,63$) und E/P ($H_e = 0,62$) festgestellt, etwas geringere Werte in den Gruppen F/G/NL ($H_e = 0,55$) und N/S ($H_e = 0,51$) und die niedrigsten Werte innerhalb UK ($H_e = 0,42$) und CZ ($H_e = 0,37$). Bei Betrachtung der Allelfrequenzen ist die gleiche Reihenfolge erkennbar, beginnend mit den höchsten Werten für A/CH (2,92), E/P (2,88) und F/D/NL (2,56) gefolgt von N/S (2,3), UK (2,01) und CZ (1,84). Analog wurde der größte Anteil seltener Allele mit 27,5% in der E/P-Gruppe festgestellt und der niedrigste Anteil in der CZ-Gruppe (3%).

Basierend auf diesen Ergebnissen ist insbesondere der Erhaltung bereits bestehender genetischer Ressourcen und der Variabilität erhöhte Aufmerksamkeit zu widmen und der Austausch genetischen Materials zu
fördern, um eine optimale Nutzung der insgesamt vorhandenen genetischen Variation zu ermöglichen.


Hinsichtlich der Beziehung der genannten Parameter zueinander konnten bei der DH-Population keine ausgeprägte Korrelationen der Stängelfestigkeit mit dem Stängeldurchmesser ($r = 0,143, P <0,05$) und der Halmwandstärke ($r = 0,196, P <0,05$) sowie dem Markdurchmesser ($r = -0,064$) festgestellt werden. Eine engere positive Korrelation liegt zwischen Stängeldurchmesser und Markdurchmesser ($r = 0,529, P <0,001$) bzw. Halmwandstärke ($r = 0,259, P <0,01$) vor. Deutlich negativ korreliert sind Markdurchmesser und Halmwandstärke ($r = -0,682, P <0,001$).

Nach einem Screening von 771 SSR-Markern konnten 200 zwischen den beiden Elternlinien ‘CA9613’ und ‘H1488’ polymorphe Marker identifiziert werden, die im weiteren für die Erstellung einer genetischen Karte anhand der

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9 LIST OF ABBREVIATIONS

A allelic richness
AFLPs amplified fragment length polymorphisms
AMOVA analysis of molecular variance
ANOVA analysis of variance
BC backcross
CIM composite interval mapping
cM centi Morgan
COP coefficient of parentage
CTAB cetytrimethylammonium bromide
CV coefficient variation
CWT culm wall thickness
CZ Czech republic
DH doubled haploid
DNA deoxyribonucleic acid
dNTP deoxyribonucleotide triphosphate
E/P Spain/Portugal
EDTA ethylenediamine tetraacetic acid
ESTs expressed sequence tags
F/G/NL France/Germany/Netherlands
g gram
GCA general combining ability
GS genetic similarity
h hour
H\(_e\) gene diversity
I litre
I/D insertion/deletion
IAM infinite allele model
LD linkage disequilibrium
w/v weight/volum
LOD logarithm of the odds
LIST OF ABBREVIATIONS

LR likelihood ration
m metre
MAS marker-assisted selection
min minute
mm millimeter
PCR polymerase chain reaction
PD pith diameter
PIC polymorphism information content
QTL quantitative trait loci
RAPDs random amplified polymorphic DNAs
RFLPs restriction fragment length polymorphisms
RILs recombinant inbred lines
s second
SCA specific combining ability
SD stem diameter
SIM simple interval mapping
SIMQUAL similarity of qualitative data
SNPs single nucleus polymorphisms
SS stem strength
SSD single seed descent
SSRs simple sequence repeats
UK the United Kingdom
UPGMA unweighted pair-group method with arithmetic average
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11 DECLARATION

I hereby declare that the thesis entitled “Analysis of Genetic Diversity among Current Spring Wheat Varieties and Breeding for Improved Yield Stability of Wheat (Triticum aestivum)” 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.

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(Lin Hai)