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Plant genetic resources - a prerequisite for drought tolerance breeding in cereals

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Abstract

One of the largest *ex situ* genebanks of the world is located at the Leibniz Institute of Plant Genetics and Crop Plant Research in Gatersleben. This collection comprises wild and primitive forms, landraces as well as old and more recent cultivars of cultivated plants including cereals. Wheat is the major crop having almost 30,000 accessions. Beside the long term storage and frequent regeneration of the material phenotypic characterisation and evaluation data are collected as a prerequisite for gene identification and mapping. In our presentation we give examples for the successful utilisation of germplasm for the identification of genes (quantitative trait loci) determining drought tolerance in wheat. The material was investigated at the seedlings and adult plant (grain filling) stages.

Keywords

Drought tolerance, germplasm, osmotic stress, QTL mapping, *Triticum*, wheat

Ex situ genebank collections

Plant *ex situ* genebank collections comprise seed genebanks, field genebanks and *in vitro* collections. Species whose seed can be dried, without damage, down to low moisture contents, can be stored in seed banks. Field genebanks and *in vitro* storage are used primarily for species which are either vegetatively propagated or which have recalcitrant seeds that cannot be dried and stored for long periods. It is estimated that worldwide approximately 90% of the genebank holdings are stored as seeds whereas around 10% and 1% are maintained *in vivo* in the field and *in vitro*, respectively (FAO 1998, BÖRNER 2006).

Globally germplasm collections contain more than 6 million accessions of plant genetic resources. Wheat represents the biggest group with about 800,000 accessions followed by barley (490,000 accessions) and rice (420,000 accessions). The 10 largest world-wide germplasm collections by crop are given in *Table 1* (FAO 1998).

The German *ex situ* genebank, located at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany, is one of the largest global collections. About 150,000 accessions are maintained including cereals (65,000), legumes (28,000), vegetables (18,000), forage crops (14,000), oil crops (8,000), potatoes (6,000) and medicinal and spice plants (6,000). Within the cereals wheat (*Triticum*) and barley (*Hordeum*) represent the largest groups having 28,000 and 22,000 accessions, respectively (*Figure 1*, ANONYMUS 2008). The Gatersleben genebank holdings consist of plant materials collected as early as the 1920's. Initial collection missions were made to the Austrian Alps (1922-1932), Anatolia (1928-1932), the Hindukush (1935), the Himalayas (1937-38), Tibet (1938-39), Ethiopia and Eritrea (1937-1939) and the Balkans (1941-1942) (BÖRNER 2006).

Table 1: **List of the ten largest world-wide germplasm collections by crop (FAO 1998)**

Crop	Genus	Accessions
Wheat	*Triticum*	788,654
Barley	*Hordeum*	486,724
Rice	*Oryza*	420,341
Maize	*Zea*	261,584
Bean	*Phaseolus*	268,369
Oat	*Avena*	223,287
Soybean	*Glycine*	176,400
Mustard	*Brassica*	106,923
Sorghum	*Sorghum*	168,550
Apple	*Malus*	97,543

Beside the collection and maintenance of the germplasm in genebanks one of the main challenges nowadays is the evaluation and successful utilisation of these resources.

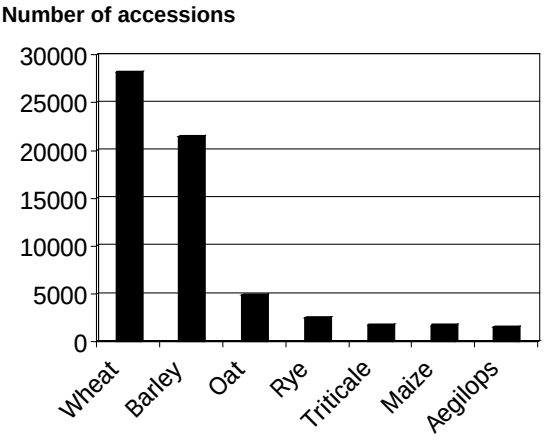

Figure 1: **Inventory of the cereals collection maintained in the German *ex situ* genebank (ANONYMUS 2008)**

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Phenotypic characterisation associated with genetic analyses will enable us to the potential of genebank accession for further improvement of modern cultivars.

Drought tolerance at the seedlings stage

A set of 114 recombinant inbred lines (RILs) from the International Triticeae Mapping Initiative (ITMI) mapping population was used for detecting genes/quantitative trait loci (QTL) for drought (osmotic stress) tolerance at the seedlings stage. This population was derived by single seed descent F8 from the cross between W 7984 and Opata 85. The parent W 7984 is an amphihexaploid wheat synthesized from *Triticum tauschii* (DD) × *Triticum durum* (AABB) cv. Altar 84, while Opata 85 is a Mexican spring wheat variety developed at CIMMYT (Centro Internacional de Mejoramiento de Maiz y Trigo) in Mexico.

Osmotic stress was induced by 12% PEG (PEG 6000) treatment. Distilled water was used as control. Eight seeds per line and variant were placed in lanes in covered transparent plastic boxes on two layers of filter paper moistened with PEG solution or distilled water. Seeds were germinated in a growth chamber at 21±1°C in dark for 3 days and under 12 h light:12 h dark regime in the next 5 days. Root length, coleoptile length and shoot length were measured on 5 seedlings per line. Root length/shoot length ratio was also determined. For all traits the tolerance index was calculated as a ratio between the mean trait value obtained under stress and the corresponding trait value under control. Three independent replications of the experiment were conducted.

The phenotypic data were used for QTL analysis. The presence of QTL was determined with QGene software package (NELSON 1997) using single marker analysis. In total of 35 regions on 10 chromosomes contributed effects on seedling growth traits. Five of the chromosomes are presented in *Figure 2*. The details of the study are described in LANDJEVA et al. (2008).

Drought tolerance at the adult plant stage

The same set of 114 RILs of the ITMI population was grown on the experimental fields at IPK Gatersleben during the growing seasons 2001 and 2003. The lines were grown together with the parents in plots with four rows, 1 m long with 20 cm between rows. Anthesis was recorded when about 50% of the plants showed spikes with exerted anthers in the central third of the spikes. Fourteen days after anthesis, chemical desiccation was applied to two rows of the plot of each genotype, while the other two rows were kept untreated (without desiccation). The desiccation treatment was applied by spraying the whole plant canopy to full wetting with an aqueous solution of potassium iodide (KI, 0.5% w/v). The desiccant was applied using a hand-held boom sprayer allowing spray penetration to the whole plant canopy.

At maturity, the spikes were collected and threshed on a plot basis. After harvest, 1000-grain weight was measured for treated and non-treated rows. Based on that, the post-anthesis drought tolerance index was calculated from the performance of the stressed plants (S) relative to its respective non-stressed controls (C) within the same replicate and cal-

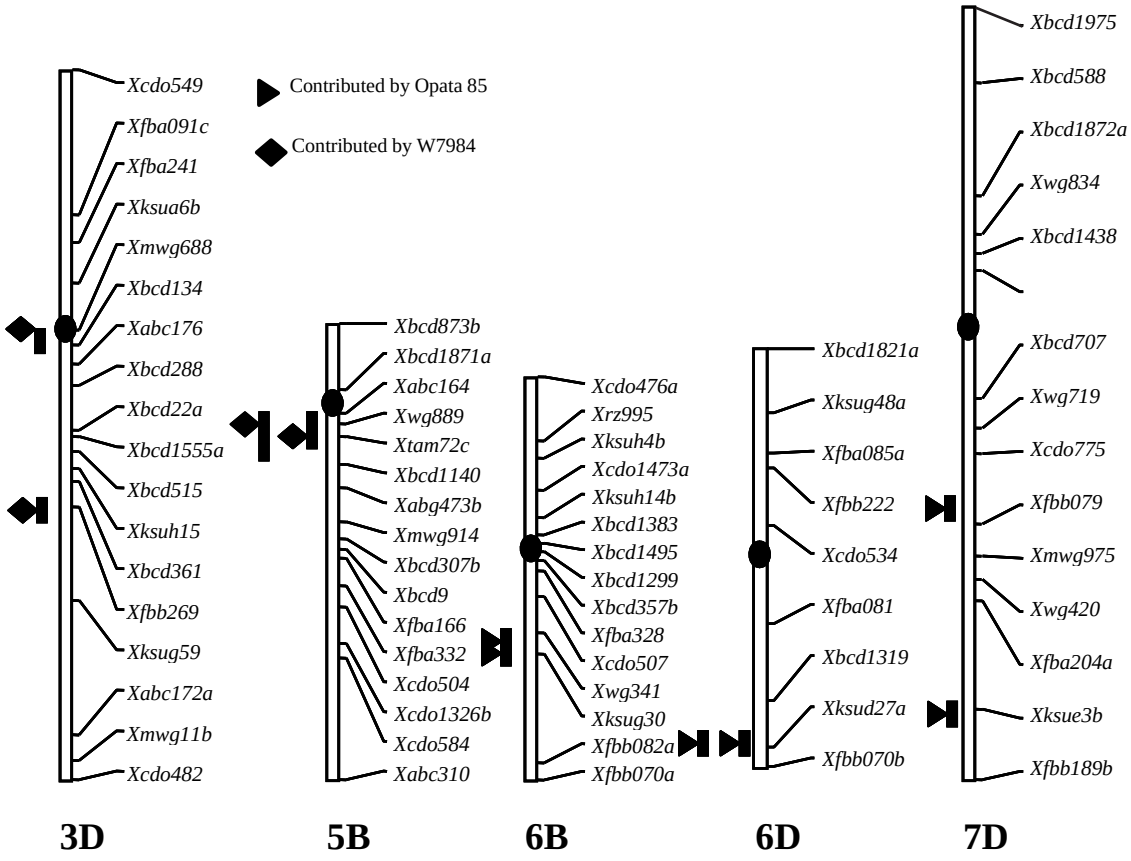

Figure 2: **Selected chromosomes carrying loci determining seedling growth traits (modified after LANDJEVA et al. 2008)**

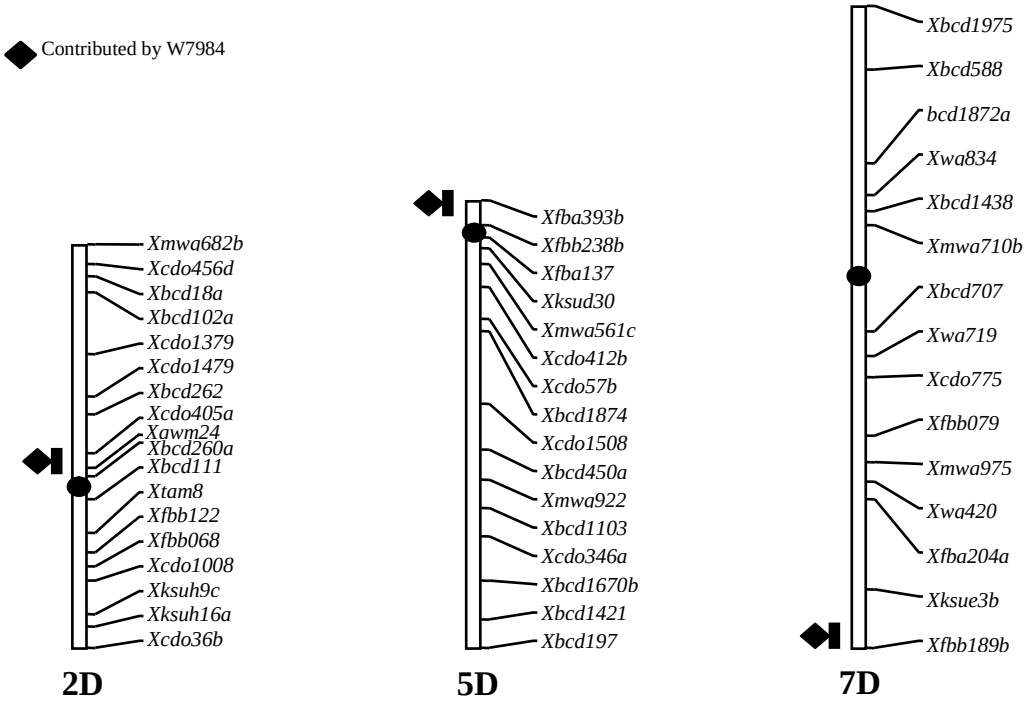

Figure 3: **Chromosomes carrying loci determining post anthesis drought tolerance/stem reserve mobilisation (SALEM et al. 2007)**

culated as percentage according to BLUM et al. (1983a,b). The stress tolerance index (STI) was calculated as

$$STI(\%) = \frac{S \times 100}{C}$$

where C is the 1000-grain weight under control and S the 1000-grain weight under potassium iodide (KI) treatment.

Using the QGene software package (NELSON 1997) QTL for stem reserves mobilisation (drought tolerance after anthesis), were mapped on chromosomes 2D, 5D and 7D. Results are presented in *Figure 3*, details are described by SALEM et al. (2007). Interestingly, the QTL on chromosome 7DL maps in a comparable position to one being responsible for osmotic stress tolerance at the seedlings stage (*Figures 2* and *3*). In addition, it should be mentioned that clusters of QTLs determining grain yield under drought stress were detected in homoeologous regions at distal parts of chromosomes 7AL and 7BL by QUARRIE et al. (2005).

Conclusions

Extensive germplasm collections do exist globally in which wheat represents the largest crop. Nowadays the utilisation of the genebank holdings for crop improvement including abiotic stress tolerance is one of the main challenges. The successful exploitation of genetic resources requires (1) extensive characterisation and evaluation and (2) genetic analysis and molecular mapping of the relevant traits. Detected loci determining drought tolerance at different developmental stages can be combined and transferred to modern cultivars via marker assisted selection.

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