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REVIEWS  
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## *Ex-situ* Genebanks—Seed Treasure Chambers for the Future

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**Abstract**—The paper gives an overview about the past and present situation of the maintenance of plant genetic resources in *ex-situ* genebanks where seed storage is the prevailing way of conservation. Therefore, seed storability/longevity is of exceptional importance for germplasm conservation. Beside environmental influence on the trait a strong genetic component was proven. Genetic analyses performed at IPK Gatersleben on barley, wheat, oilseed rape and tobacco are summarized. It was demonstrated that seed response to ageing treatment appears to be significantly influenced by both genetic background and maternal environment. It was also shown that processes involved in the experimental ageing protocols (high temperature and humidity) only partly mirror those operating during long term genebank storage.

**Keywords:** plant genetic resources, genebank, seed longevity, germplasm, conservation, genetic analysis—QTL

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### INTRODUCTION

About 100 years ago plant biologists started to recognize the potential of plant diversity and the necessity to preserve it. In February 1914 the German geneticist E. Baur gave a presentation at a seed conference organized in Berlin ending with the statement: ‘It is very urgent now to become active to save and maintain the quickly disappearing old and primitive varieties of our cultivated crops’ [1].

First documented germplasm collection missions to Pamir and Iran, however, were organized by N.I. Vavilov in 1916. Four years later N.I. Vavilov became the director of the Department of Applied Botany in Petrograd. Between 1920 and 1940 no less than 140 expeditions were sent out within the Soviet Union and 40 expeditions all over the world, visiting 64 different countries. By 1940 the collection of cultivated plants amounted 200,000 accessions [2].

Collection missions initiated by German scientists date back to the 1930s and had been carried out by E. Baur and R. Schick in South America in 1930–1931, by A. Scheibe in the Hindukush (1935–1936), by C. Troll and R. Schottenloher in Ethiopia and Eritrea (1937–1938) and by E. Schäfer in Tibet (1938–1939). Since that time the establishment of an institute for research on crop plants with a mandate to conserve germplasm was planned, however, the outbreak of the Second World War delayed the foundation until April 1, 1943. As the first director of the Institute of Crop

Plant Research Hans Stubbe was appointed. The institute was originally founded near Vienna but was established in Gatersleben by the end of 1945 [3].

There was a close interaction between Russian and German scientists going back to 1921 when N.I. Vavilov visited Germany meeting E. Baur and Th. Roemer. Further communications took place at the 5th International Genetics Congress in Berlin, in September 1927 and at the 1st All Union Congress on Genetics, Plant, Seed and Animal Breeding in Leningrad in January 1929 [4]. There was a significant influence of N.I. Vavilov on German scientists concerned with the conservation of cultivated plants and wild relatives [5].

Beside the pioneering activities initiated by the Russian and German Scientists other countries did follow the idea to create national facilities for preserving plant genetic resources. In 1958 a National Seed Storage Laboratory came into operation in Fort Collins, USA. Since then, many more seedbanks have been established all over the world. By 1985, it was estimated that there were 227 national genebanks in over one hundred countries and international centres storing seeds of crop plants and wild species. In 1992 the number of germplasm collections amounts 993 in 121 countries holding 3.6 million accessions [6].

### PRESENT SITUATION

At present, regarding to the latest FAO report on the state of the world’s plant genetic resources for food

**Table 1.** The six largest national germplasm collections on earth according FAO [7]

Institution	Country	Accessions
NPGS (National Plant Germplasm System)	USA	508,994
ICGR-CAAS (Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Science)	China	391,919
NBPGR (National Bureau of Plant Genetic Resources)	India	366,333
VIR (Vavilov All-Russian Institute of Plant Genetic Resources)	Russia	322,238
NIAS (National Institute of Agrobiological Science)	Japan	243,463
IPK (Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung)	Germany	148,128

**Table 2.** World-wide germplasm collections with more than 100,000 accessions by crop according FAO [7]

Crop	Genus	Accessions
Wheat	<i>Triticum</i>	856,168
Rice	<i>Oryza</i>	773,948
Barley	<i>Hordeum</i>	466,531
Maize	<i>Zea</i>	327,932
Bean	<i>Phaseolus</i>	261,963
Sorghum	<i>Sorghum</i>	235,688
Soybean	<i>Glycine</i>	229,944
Oat	<i>Avena</i>	130,653
Groundnut	<i>Arachis</i>	128,435
Cotton	<i>Gossypium</i>	104,780

and agriculture about 1750 *ex situ* genebanks globally are housing 7.4 million accessions [7]. Of this amount, national genebanks conserve about 6.6 millions. The remaining 800,000 accessions are maintained in international CGIAR (Consultative Group on International Agricultural Research) genebanks comprising 11 institutions. Both Russia and Germany contain the two largest national collections within Europe and are among the top six in the world (Table 1). Considering major crop groups about 45 percent of all the accessions in the world's genebanks are cereals, followed by legumes (15 percent), fruits and forage crops (each 6–9 percent) as well as roots and tubers, oil crops and fibre crops (each 2–3 percent). The largest total numbers per crop are covered by wheat and rice comprising 1.6 million accessions. Crops with more than 100,000 accessions stored in the global genebanks are listed in Table 2. It is estimated that 90% of all genebank holdings are stored as seeds whereas less than 10% and less than 1% are maintained *in vivo* (field genebanks) and *in vitro* (tissue culture and cryo preservation), respectively [8].

In February 2008, the Svalbard Global Seed Vault (SGSV) was opened to become a backup facility for the world's seed banks. The Seed Vault is managed in partnership by the Government of Norway, the Nordic Genetic Resource Center (NordGen) and the Global Crop Diversity Trust (the Trust). NordGen is

a public regional institute supported by the governments of the Nordic countries, and the Trust is an independent international organization based in Bonn, Germany. The Norwegian Ministry of Agriculture and Food is the legally responsible authority for the Seed Vault, and its operation is overseen by an International Advisory Council. The Seed Vault provides free-of-charge, long-term storage of duplicates from genebanks around the world and works as an insurance policy against incremental or catastrophic loss of the original collections. At this moment, the total safety back-up collection comprised 983,524 accessions belonging to 1091 genera and 6005 species. Depositors are 76 institutions from all over the world [9, 10].

Following the Genebank Standards for Plant Genetic Resources for Food and Agriculture [11], before storage seeds should be dried to equilibrium in a controlled environment of 5–20°C and 10–25 percent of relative humidity (RH), depending upon species. After drying, all seed samples need to be sealed in a suitable airtight container for long-term storage. Most-original-samples and safety duplicate samples should be stored under long-term conditions (base collections) at a temperature of  $-18 \pm 3^\circ\text{C}$  and  $15 \pm 3\%$  RH. For medium-term conditions (active collections), samples should be stored under refrigeration at  $5-10^\circ\text{C}$  and  $15 \pm 3\%$  RH. In addition the document is

providing standards for acquisition of germplasm, seed viability monitoring, regeneration, characterization, evaluation, documentation, distribution and exchange, safety duplication and security as well as personnel.

More recently molecular tools (genomics) are used to genotype whole collections. At the IPK in Gatersleben genome-wide genotyping-by-sequencing was performed for 21,500 barley accessions maintained in the genebank [12]. The data provides new insights into the global population structure of the whole collection and points out redundancies but also coverage gaps.

## SEED LONGEVITY

Clearly, seed storage is the predominant mode of plant genetic resources conservation (90%) and, therefore, studies on seed longevity are of special importance for germplasm preservation. For this reason, at IPK in Gatersleben research was initiated for a range of crops stored in the genebank over decades. Variation between crop species was detected for seeds stored at around 20°C and 50% RH [13]. Pea (*Pisum sativum* L.) and common bean (*Phaseolus vulgaris* L.) retained the viability over the longest period (29 and 21 years, respectively) whereas chive (*Allium schoenoprasum* L.) seeds survived for only five and lettuce (*Lactuca sativa* L.) for seven years. The results presume that the predominant seed storage compounds may affect seed longevity with proteins (legumes) > carbohydrates (cereals) > lipids (oil crops).

Intraspecific variation of genebank collections stored between 26 and 33 years at 0°C was investigated by [14]. Crops showed high germination when germinated within 5 years post harvest, but germination of most accessions within species separated strongly after 20 years. In particular, wheat (*Triticum aestivum* L.) germination resulted between 0 and 87% after 34 years of storage whereas barley (*Hordeum vulgare* L.) accessions germinated between 43 and 95% after 35 years. Because the accessions within the species under investigation were regenerated and harvested in the same year, the same harvest (threshing and cleaning) technologies were applied and the storage conditions were identical it can be concluded that the differences in germination after long term storage are genetically based. In consequence, at IPK we started to perform genetic studies on seed longevity.

## GENETICS ON SEED LONGEVITY

### Barley

In barley three bi-parental doubled haploid (DH) mapping populations have been investigated in parallel: 'Steptoe' × 'Morex' (150 DH lines, spring growth habit), 'OWB<sub>DOM</sub>' × 'OWB<sub>REC</sub>' (94 DH lines, spring growth habit) and 'Angora' × 'W704/137' (100 DH lines, winter growth habit) [15]. Artificial aging was performed at 43°C and 100% RH for 72 h and as a sec-

ond treatment for at 44°C and 18% seed moisture content (SMC) for 72 h. In total (3 mapping populations, two treatments) four major QTLs on chromosomes 2H, 5H (two loci) and 7H were detected. The locus on chromosome 7H was detected in the 'OWB<sub>DOM</sub>' × 'OWB<sub>REC</sub>' and 'Angora' × 'W704/137' (two ageing treatments) populations in the region of the gene *nud* controlling the character naked/hulled caryopsis for which the DH lines did segregate for. Because the hulled parents contributed to increased seed longevity it was concluded that the gene itself may be involved. The chromosome 2H locus was discovered in the region of the dwarfing gene *Zeo1* determining a short plant habit with compact spikes and segregating in the mapping population 'OWB<sub>DOM</sub>' × 'OWB<sub>REC</sub>'. The high longevity was provided by the parent having the wild-type allele determining the normal growth type. A negative effect of the changed compact spike architecture was suggested. Two of the mapping populations were saturated with expressed sequence tags and, therefore, a sequence homology search was performed to derive putative functions of the genes linked with the longevity QTLs. Proteins playing an important role in the regulation of biotic and abiotic stresses or the inflorescence architecture were identified.

Furthermore, an association mapping panel consisting of 175 landraces and cultivars of barley was examined [16]. Seeds were obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA) [17]. The panel was grown in the same growing season but on two different field sites separated by less than 800 m. One year after harvest seeds were subjected to two artificial ageing regimes (43°C, 100% RH for 72 h and 45°C, 60% RH for 15 days). Genome-wide association mapping revealed 107 marker trait associations (MTAs). Beside common MTAs (loci) many 'field specific' loci were detected. Due to the identical regeneration year and the close proximity of the experiments, it was concluded that soil composition may have been one reason responsible for the differences. This was supported by a soil analysis, indicating a difference in nutrient contents, mainly phosphorus, potassium, manganese, and nitrogen.

### Wheat

In wheat [18] published a study on a set of 85 bread wheat (*Triticum aestivum* L.) cv. 'Chinese Spring'/*Aegilops tauschii* Coss.—the D genome progenitor—introgression lines where D genome-wide segments of the *Triticum aestivum* genome were substituted by its homoeologues from *Aegilops tauschii* [19]. The introgression lines population was investigated for a range of seedling traits including longevity. Artificial ageing was performed at 43°C and 100% RH for 72 h. The authors identified two sections in the centromere regions of chromosomes 1DS and 5DL. The donor of

the favourable alleles was the cultivated wheat 'Chinese Spring' but not the wild species *Aegilops tauschii*.

Recombinant inbred lines (RILs) of the International Triticeae Mapping Initiative (ITMI) mapping population regenerated in two different seasons were investigated by [20]. This population was derived from a cross between the bread wheat cultivar 'Opata 85' and the 'synthetic' hexaploid wheat 'W7984.' The parents of 'W7984' were the durum wheat cultivar 'Altar 84' and the *Aegilops tauschii* accession 'CIGM86.940' [21]. Seeds of 86 and 99 RILs originated from the two different growing seasons were used for two experimental ageing treatments (43°C, 100% RH, 72 h; 43°C, 18% SMC, 72 h). Contradictory QTL were detected, indicating that growing and/or storage conditions as well as that seed moisture content during the experimental ageing treatment process exert a large influence on trait mapping. In addition the authors did investigate an association mapping panel comprising 96 winter wheat accessions from 21 countries. It was assembled at the Institute of Field and Vegetable Crops, Novi Sad, Serbia [22]. Accessions were selected on the basis of their phenotypic diversity with respect to a group of key agronomic traits. More detailed information about the panel is given by [23]. For experimental ageing two treatments (43°C, 100% RH, 72 h; 43°C, 18% SMC, 72 h). were applied. Association mapping revealed 73 markers associated with longevity on 14 chromosomes. Most of the loci were treatment specific.

Another panel covering 183 bread wheat (129 spring type, 54 winter type) accessions was studied by [24]. Seeds under investigation were taken from the genebank collection maintained at the IPK and last multiplied 1974. These were the oldest seed lots available in the storage. The grain has been stored at 0°C and the SMC maintained at  $8 \pm 2\%$ . Germination test was performed after 34 years of storage. The panel was grown out to produce fresh material. The fresh seeds were aged experimentally with two treatments (43°C, 100% RH, 72 h; 43°C, 18% SMC, 72 h). The analysis of the long term stored seeds revealed 103 MTAs whereas after experimental ageing, respectively, 74 and 97 associations were identified. It was shown that there are distinct sets of genomic regions harbouring loci active during the process of natural and induced ageing. This clearly indicates that the mechanisms involved in the experimental ageing protocols do not fully mirror those operating during long term low temperature storage, although the sharing of some genomic regions does imply that experimental ageing can to a certain extent be predictive. Synteny of longevity loci between wheat, *Aegilops*, barley and rice are given in Fig. 1.

A further bi-parental population of 82 RILs created from a cross between winter wheat cultivars 'History' and 'Rubens' [25] was studied by [26]. Experimental ageing was performed at 45°C and 60% RH for 19 days.

One putative major QTL was detected on chromosome 1AL. On the same chromosome arm, loci for seed longevity were reported earlier [20, 24]. Whether some of them might correspond to the locus detected in the 'History' × 'Rubens' mapping population can only be speculated.

Beside hexaploid wheat, very recently a study in tetraploid durum wheat (*Triticum durum* Desf.) was initiated [27]. Experimental ageing was performed (43°C, 100% RH, 72 h) using a bi-parental mapping population (98 RILs) derived from a cross between the variety 'Omrabi' and a breeding line 'Belikh' [28]. Several QTL were detected on six different chromosomes.

#### *Oil Seed Rape*

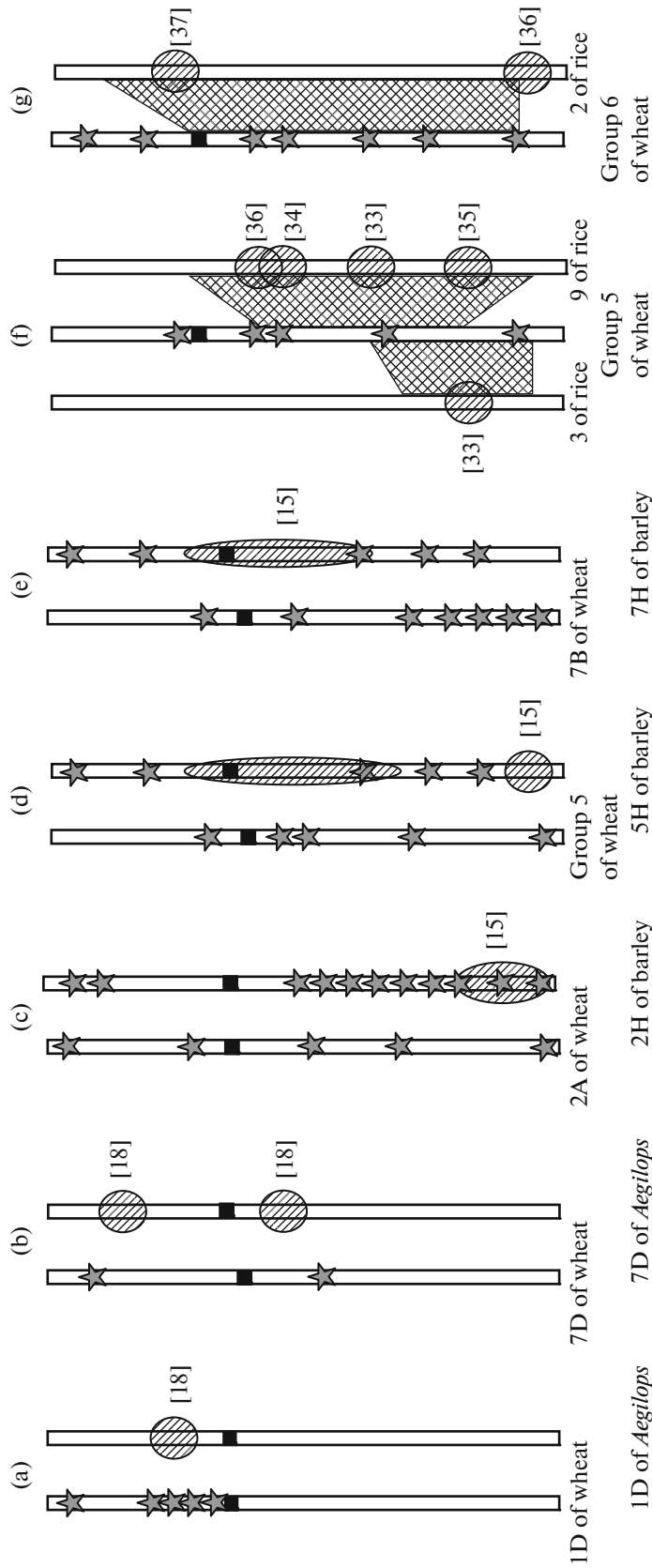
Three different experimental ageing protocols (44°C, 99% RH, 48 h; 42°C, 99% RH, 48 h; 45°C, 60% RH, 17 days) were applied to a population of 153 DH lines of the winter oilseed rape YE2-DH mapping population to explore the genetic basis of seed longevity [29, 30]. In total 14 significant QTL affecting seed longevity were identified on seven different chromosomes, of which 13 were method-specific. It was surprising that even a modest increase in ageing temperature (from 42 to 44°C) can have a major effect on the expression of relevant genes.

#### *Tobacco*

One last example in this review is given for tobacco [31]. A population of 122 RILs derived from a cross between the cultivars 'Florida 301' and 'Hicks' was studied [32]. Experimental ageing was performed at 45°C and 60% RH for 30 days. QTL mapping revealed three genomic regions located on three different linkage groups to be associated with the germination after ageing treatment. The parent 'Florida 301' donated a favorable allele for germination speed after ageing treatment on linkage group seven. Interestingly, the position of this locus compared well with a QTL detected in the same population, in a former study examining resistance against the black shank disease caused by *Phytophthora nicotianae* where 'Florida 301' was the disease resistant parent [32]. Above-ground symptoms of the disease are wilting and yellowing of the leaves that can have a pronounced effect on seed development.

### CONCLUSIONS

Worldwide 7.4 Million accessions are maintained in *ex-situ* genebanks. The predominant way of conservation is storing seeds at low moisture content and low temperature. The storability/longevity of the seeds depends on the storage environment but is also species specific. In addition there is an intraspecific variation for seed longevity which is genetically determined.



**Fig. 1.** Synteny of longevity loci between (a) chromosomes 1D of wheat and 1D of *Aegilops*, (b) 7D of wheat and 7D of *Aegilops*, (c) chromosomes 2A of wheat and 2H of barley, (d) homologous group 5 of wheat and chromosome 5H of barley, (e) 7B of wheat and 7H of barley, (f) homologous group 5 of wheat and chromosomes 3 and 9 of rice and (g) homologous group 6 of wheat and chromosome 2 of rice. Small squares indicate centromeres on wheat and barley. Stars on wheat chromosomes represent areas in association with longevity detected in [24] and on barley detected in [16]. Shaded circles indicate seed longevity QTLs identified in previous studies either in barley or rice. [38] is used to compare wheat with rice. The figure is modified from [24].

The inheritance of the trait is quantitatively. Numerous quantitative trait loci were detected in several species. Results presented here clearly demonstrate that already the environmental conditions during the regeneration of the seeds under investigation have a significant effect on seed longevity.

Most of the studies on seed longevity were performed using experimental ageing methods applying increased temperatures and moisture contents. Comparing those results with data obtained from genetically identical material but stored under cold storage conditions in the genebank, different genomic regions responsible for the storability of the seeds were detected. Therefore, instead of experimental ageing, future investigations on seed longevity should focus on material aged under long term cold storage conditions in order to get more realistic information for increasing the efficiency of germplasm conservation.

#### COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

#### REFERENCES

- Baur, E., Die Bedeutung der primitiven Kulturrassen und der wilden Verwandten unserer Kulturpflanzen für die Pflanzenzüchtung, *Jb. Deut. Landw.-Ges.*, 1914, vol. 29, pp. 104–109.
- Rodin, L.E., Reznik, S., Stapleton, P., and Löve, D., *Five Continents by N. I. Vavilov*, 2010.
- Stubbe, H., Geschichte des Instituts für Kulturpflanzenforschung Gatersleben der Deutschen Akademie der Wissenschaften zu Berlin (1943–1968), in *Studien zur Geschichte der Akademie der Wissenschaften der DDR*, 1982, vol. 10.
- Esakov, V.D., On the scientific relations of N.I. Vavilov to German geneticists and breeders, *Die Kulturpflanze*, 1988, vol. 36, pp. 61–69.
- Börner, A., Nickolai Ivanovich Vavilov and his footprint on plant genetic resources conservation in Germany, *S.-kh. Biol.*, 2012, vol. 5, pp. 20–30.
- Chen, H.F., Seedbanks: conserving the past for the future, *Seed Sci. Technol.*, 1994, vol. 22, pp. 385–400.
- Commission on Genetic Resources for Food and Agriculture, *The Second Report on the State of the World's Plant Genetic Resources for Food and Agriculture*, Rome: Food and Agriculture Organization of the United Nations, 2010.
- Food and Agriculture Organization of the United Nations, *The State of the World's Plant Genetic Resources for Food and Agriculture*, Rome, 1998.
- Westengen, O.T., Jeppson, S., and Guarino, L., Global *ex-situ* crop diversity conservation and the svalbard global seed vault: assessing the current status, *PLoS One*, 2013, vol. 8, e64146.
- <https://www.nordgen.org/sgsv/>.
- Food and Agriculture Organization of the United Nations, *Genebank Standards for Plant Genetic Resources for Food and Agriculture*, Rome, 2014, rev. ed.
- Milner, S.G., Jost, M., Taketa, S., et al., Genebank genomics highlights the diversity of a global barley collection, *Nat. Genet.*, 2019, vol. 51, pp. 319–326. <https://rdcu.be/bbNJu>.
- Nagel, M. and Börner, A., The longevity of crop seeds stored under ambient conditions, *Seed Sci. Res.*, 2010, vol. 2, pp. 1–20.
- Nagel, M., Rehman-Arif, M.A., Rosenhauer, M., and Börner, A., Longevity of seeds—intraspecific differences in the Gatersleben genebank collections, in *Tagungsband 60: Tagung der Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs, Gumpenstein, Österreich, 24–26 November 2009*, 2010, pp. 179–181.
- Nagel, M., Vogel, H., Landjeva, S., et al., Seed conservation in *ex situ* genebanks—genetic studies on longevity in barley, *Euphytica*, 2009, vol. 170, pp. 5–14.
- Nagel, M., Kranner, I., Neumann, K., et al., Genome-wide association mapping and biochemical markers reveal that seed ageing and longevity are intricately affected by genetic background, developmental and environmental conditions in barley, *Plant Cell Environ.*, 2015, vol. 38, pp. 1011–1022.
- Varshney, R.K., Paulo, M.J., Grando, S., et al., Genome wide association analyses for drought tolerance related traits in barley (*Hordeum vulgare* L.), *Field Crops Res.*, 2012, vol. 126, pp. 171–180.
- Landjeva, S., Lohwasser, U., and Börner, A., Genetic mapping within the wheat D genome reveals QTL for germination, seed vigour and longevity, and early seedling growth, *Euphytica*, 2010, vol. 171, pp. 129–143.
- Pestsova, E.G., Börner, A. and Röder, M.S., Development and QTL assessment of *Triticum aestivum*—*Aegilops tauschii* introgression lines. *Theor. Appl. Genet.*, 2006, vol. 112, pp. 634–647.
- Rehman Arif, M.A., Nagel, M., Neumann, K., et al., Genetic studies of seed longevity in hexaploid wheat using segregation and association mapping approaches, *Euphytica*, 2012, vol. 186, pp. 1–13.
- Börner, A., Schumann, E., Fürste, A., et al., Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.), *Theor. Appl. Genet.*, 2002, vol. 105, pp. 921–936.
- Quarrie, S.A., Dodig, D., Pekic, S., et al., Prospects for marker-assisted selection of improved drought responses in wheat, *Bulg. J. Plant Physiol.*, 2003, special issue, pp. 83–95.
- Neumann, K., Kobiljski, B., Dencic, S., et al., Genome-wide association mapping: a case study in bread wheat (*Triticum aestivum* L.), *Mol. Breed.*, 2011, vol. 27, pp. 37–58.
- Rehman Arif, M.A., Nagel, M., Lohwasser, U., and Börner, A., Genetic architecture of seed longevity in bread wheat (*Triticum aestivum* L.), *J. Biosci.*, 2017, vol. 42, pp. 81–89.
- Holzappel, J., Voss, H.-H., Miedaner, T., et al., Inheritance of resistance to *Fusarium* head blight in three European winter wheat populations, *Theor. Appl. Genet.*, 2008, vol. 117, pp. 1119–1128.

26. Börner, A., Nagel, M., Agacka-Mołodoch, M., et al., QTL analysis of falling number and seed longevity in wheat (*Triticum aestivum* L.), *J. Appl. Genet.*, 2018, vol. 59, pp. 35–42.
27. Rehman Arif, M.A. and Börner, A., Mapping of QTL associated with seed longevity in durum wheat (*Triticum durum* Desf.), *J. Appl. Genet.*, 2019, vol. 60, pp. 33–36.
28. Nagel, M., Navakode, S., Scheibal, V., et al., The genetic basis of durum wheat germination and seedling growth under osmotic stress, *Biol. Plant.*, 2014, vol. 58, pp. 681–688.
29. Nagel, M., Rosenhauer, M., Willner, E., et al., Seed longevity in oilseed rape (*Brassica napus* L.)—genetic variation and QTL mapping, *Plant Genet. Res.: Character. Util.*, 2011, vol. 9, pp. 260–263.
30. Badani, A.G., Snowdon, R.J., Baetzel, R., et al., Co-localisation of a partially dominant gene for yellow seed colour with a major QTL influencing acid detergent fibre (ADF) content in different crosses of oilseed rape (*Brassica napus*), *Genome*, 2006, vol. 49, pp. 1499–1509.
31. Agacka-Modoch, M., Nagel, M., Doroszewska, T., et al., Mapping quantitative trait loci determining seed longevity in tobacco (*Nicotiana tabacum* L.), *Euphytica*, 2015, vol. 202 pp. 479–486.
32. Xiao, B., Drake, K., Vontimitta, V., et al., Location of genomic regions contributing to *Phytophthora nicotianae* resistance in tobacco cultivar Florida 301, *Crop Sci.*, 2013, vol. 53, pp. 473–481.
33. Xue, Y., Zhang, S.Q., Yao, Q.H., et al., Identification of quantitative trait loci for seed storability in rice (*Oryza sativa* L.), *Euphytica*, 2008, vol. 164, pp. 739–744.
34. Sasaki, K., Fukuta, Y., and Sato, T., Mapping of quantitative trait loci controlling seed longevity of rice (*Oryza sativa* L.) after various periods of seed storage, *Plant Breed.*, 2005, vol. 124, pp. 361–366.
35. Zeng, D.L., Guo, L.B., Xu, Y.B., et al., QTL analysis of seed storability in rice, *Plant Breed.*, 2006, vol. 125, pp. 57–60.
36. Miura, K., Lyn, S.Y., Yano, M. and Nagamine, T., Mapping quantitative trait loci controlling seed longevity in rice (*Oryza sativa* L.), *Theor. Appl. Genet.*, 2002, vol. 104, pp. 981–986.
37. Li, G., Na, Y.W., Kwon, S.W., and Park, Y.J., Association analysis of seed longevity in rice under conventional and high-temperature germination conditions, *Plant Syst. Evol.*, 2014, vol. 300, pp. 389–402.
38. Stein, N., Prasad, M., Scholy, U., et al., A 1000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics, *Theor. Appl. Genet.*, 2007, vol. 114, pp. 823–839.