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REPORTS OF THE COORDINATORS

Overall coordinator's report

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Since the latest overall coordinator's report not too many news have happened. This year one research report was received and became included in this volume. Once again I want to stress the importance of publishing short research notes after having been published in high level journals. The barley community should gain of an overview what different barley research groups are working on and receiving new results to investigate the whole barley genome.

In this volume, BGN 44, again one hundred and thirteen barley stock descriptions are described, revised or updated with latest research results and citied literature. They are listed in table 1, pp 4-8, additionally also tables 2 and 3, pp 9-50 with BGS numbers in order (table 2) and in alphabetic order of the recommended locus names and symbols (table3) are again published to make it easy for barley researchers to find gene descriptions.

Unhappily, the construction of the 'International Database for Barley Genes and Barley Genetic Stocks' has not proceeded as promised last year because of different circumstances. The IT department at NordGen, Alnarp, Sweden, is on the way constructing a complete new lay out, I hope I can report positive information to all of you when writing the overall coordinator's report for the next volume. The purpose is to make it possible for updating descriptions directly in the electronic version.

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Coordinator's report: Translocations and balanced tertiary trisomics

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A new wheat-barley translocation line was identified as translocation involving chromosomes 5HS-7DS.7DL (Kruppa et al. 2013). Genomic in situ hybridization with D and H genomic DNA probes and fluorescence in situ hybridization with repetitive DNA probes (Afa-family, pSc119.2, and pTa71) were performed to characterize the rearranged chromosome. The effect of 5HS and the deleted 7DS fragment on the morphological traits (plant height, fertility, yield, and spike characteristics) of wheat was assessed. Despite the non-compensating nature of the translocation, the plants showed good viability. The aim of the study was to physically localize SSR markers to the telomeric and subtelomeric regions of the 7DS chromosome arm. Of the 45 microsatellite markers analyzed, ten (Xbarc0184, Xwmc0506, Xgdm0130, Xgwm0735, Xgwm1258, Xgwm1123, Xgwm1250, Xgwm1055, Xgwm1220, and Xgwm0635) failed to amplify any 7DS-specific fragments, signaling the elimination of a short chromosome segment in the telomeric region. The breakpoint of the 5HS-7DS.7DL translocation appeared to be more distal than that of reported deletion lines, which provides a new physical landmark for future deletion mapping studies.

A nice overview on available wheat/barley introgression/translocation lines has been publised by Molnar-Lang and colleauges. The meiotic pairing behaviour of wheat x barley hybrids is presented, with special regard to the detection of wheat-barley homoeologous pairing using the molecular cytogenetic techniques. The effect of in vitro multiplication on the genome composition of intergeneric hybrids is discussed, and the production and characterization of the latest wheat/barley translocation lines are presented. An overview of the agronomical traits (beta-glucan content, earliness, salt tolerance, sprouting resistance, etc.) of the newly developed introgression lines is given. The exploitation and possible use of wheat/barley introgression lines for the most up-to-date molecular genetic studies (transcriptome analysis, sequencing of flow-sorted chromosomes) are also discussed (Molnar-Lang et al. 2014).

The collection is being maintained in cold storage. To the best knowledge of the coordinator, there are no new publications dealing with balanced tertiary trisomics in barley. Limited seed samples are available any time, and requests can be made to the coordinator.

References:

Kruppa, K., A. Sepsi, E. Szakacs, M.S. Roder, and M. Molnar-Lang. **2013**. Characterization of a 5HS-7DS.7DL wheat-barley translocation line and physical mapping of the 7D chromosome using SSR markers. J Appl Genet *54*: 251-258.

Molnar-Lang, M., G. Linc, and E. Szakacs. 2014. Wheat-barley hybridization: the last 40 years. Euphytica *195*, 315-329.

Coordinator's Report: Desynaptic Genes

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The status of this genetic stock collection described in BGN 42 did not change.

No work was published describing the application of one of the desynaptic mutants. However a description of the immunological and cytological techniques developed to determine factors involved in crossover control during meiosis in barley has been published by Higgins, 2013. The immunological technique involves digesting fresh anthers followed by chromosome spreading to remove cytoplasm that causes unwanted background, whilst protecting the fragile chromosome structure from being compromised. Specific antibodies raised against meiotic proteins are then incubated with nuclei, detected with secondary antibodies conjugated to fluorescent dyes, and visualized with either wide-field or confocal microscopes. In the cytological technique, barley inflorescences are fixed, followed by dissecting out the anthers, digesting the cell walls and then spreading the meiotic chromosomes. Both techniques can be used in conjunction with specific DNA probes for fluorescent in situ hybridization (FISH) to label telomeres, centromeres, and ribosomal DNA; to identify DNA modifications such as 5-methylcytosine; and to detect the incorporation of DNA base analogues such as BrdU) or EdU to be used for a meiotic time-course or assaying newly synthesized DNA.

In addition the barley MutL Homologue (HvMLH3), a marker for class I interfering crossovers, has been characterized. Immunolocalization of HvMLH3 along with the synaptonemal complex transverse filament protein ZYP1, used in conjunction with fluorescence in situ hybridization (FISH) tagging of specific barley chromosomes, has enabled access to the physical recombination landscape of the barley cultivars Morex and Bowman. Consistent distal localization of HvMLH3 foci throughout the genome, and similar patterns of HvMLH3 foci within bivalents 2H and 3H have been observed. A difference in total numbers of HvMLH3 foci between these two cultivars has been quantified which is interpreted as representing genotypic variation in class I crossover frequency. Discrepancies between the frequencies of HvMLH3 foci and crossover frequencies derived from linkage analysis point to the existence of at least two crossover pathways in barley. It is also shown that interference of HvMLH3 foci is relatively weak compared with other plant species (Phillips et al. 2013).

Factors underlying restricted crossover localization in barley meiosis has been described (Higgins et al. 2014). Studies reviewed herein are beginning to provide an explanation for chiasma localization in barley. Moreover, they suggest a potential route to manipulating chiasma distribution that could be of value to plant breeders (Higgins et al. 2014).

References:

Higgins, J.D. 2013. Analyzing meiosis in barley. Methods Mol Biol *990*: 135-144.

Higgins, J.D., K. Osman, G.H. Jones, and F.C.Franklin. 2014. Factors underlying restricted crossover localization in barley meiosis. Annu Rev Genet *48*: 29-47.

Phillips, D., J. Wnetrzak, C. Nibau, A. Barakate, L. Ramsay, F. Wright, J.D. Higgins, R.M. Perry, and G. Jenkins, G. **2013**. Quantitative high resolution mapping of HvMLH3 foci in barley pachytene nuclei reveals a strong distal bias and weak interference. J Exp Bot *64*, 2139-2154.

Coordinator´s Report: Disease and Pest Resistance Genes

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In the table below you will find papers published in 2013/2014 extending last year´s list of information available on molecular markers for major resistance genes in barley published in Barley Genetics Newsletter 43.

List of papers published on mapped major resistance genes in barley updated until December 17, 2014.

Resistance gene	**Chromsomal location**	**Reference**
Blumeria graminis		
Ror1	1HL	Acevedo-Garcia et al. 2013
Puccinia hordei		
Rph20 *Rph20*	5HS 5HS	Singh et al. 2013 Sandhu et al. 2014
Rynchosporium commune		
$Rrs1_{Rh4}$	3H	Hofmann et al. 2013
Barley yellow dwarf virus (BYDV), Cereal yellow dwarf virus (CYDV)		
Ryd3	6HS	Lüpken et al. 2014
Barley yellow mosaic virus (BaYMV), Barley mild mosaic virus (BaMMV)		
rym4/rym5	3HL	Perovic et al. 2014
rym11	4HL	Yang et al. 2014, 2014a

References

Acevedo-Garcia, J, N.C. Collins, N. Ahmadinejad, L. Ma, A. Houben, P. Bednarek, M. Benjdia, A. Freialdenhoven, J. Altmüller, P. Nürnberg, R. Reinhardt, P. Schulze-Lefert, and R. Panstruga. 2013. Fine mapping and chromosome walking towards the *Ror1* locus in barley (*Hordeum vulgare* L.). Theor Appl Genet 126:2969-2982.

Hofmann, K, C. Silvar, A.M. Casas, M. Herz, B. Büttner, M.P. Gracia, B. Contreras-Moreira, H. Wallwork, E. Igartua, and G. Schweizer. 2013. Fine mapping of the *Rrs1* resistance locus against scald in two large populations derived from Spanish barley landraces. Theor Appl Genet 126:3091-3102.

Lüpken, T, N. Stein, D. Perovic, A. Habekuß, A. Serfling, I. Krämer, U. Hähnel, B. Steuernagel, U. Scholz, R. Ariyadasa, M. Martis, K. Mayer, R.E. Niks, N.C. Collins, W. Friedt, and F. Ordon. 2014. High-resolution mapping of the barley *Ryd3* locus controlling tolerance to BYDV. Mol Breeding 33:477-488.

Perovic, D, I. Krämer, A. Habekuß, K. Perner, R. Pickering, G. Proeseler, K. Kanyuka, and F. Ordon. 2014. Genetic analyses of BaMMV/BaYMV resistance in barley accession HOR4224 result in the identification of an allele of the translation initiation factor 4e (*Hv-eIF4E*) exclusively effective against *Barley mild mosaic virus* (BaMMV). Theor Appl Genet 127:1061-1071.

Sandhu, KS, D. Singh, and R.F. Park. 2014. Characterising seedling and adult plant resistance to *Puccinia hordei* in *Hordeum vulgare*. Ann Appl Biol 165:117-129.

Singh, N. Macaigne, and R.F. Park. 2013. *Rph20*: adult plant resistance gene to barley leaf rust can be detected at the early growth stages. Eur J Plant Pathol 137:719-725.

Yang, P, A. Habekuß, F. Ordon, and N. Stein. 2014. Analysis of bymovirus resistance genes on proximal barley chromosome 4HL provides the basis for precision breeding for BaMMV/BaYMV resistance. Theor Appl Genet 127:1625-1634.

Yang, P., T. Lüpken, A. Habekuß, G. Hensel, B. Steuernagel, B. Kilian, R. Aiyadasa, A. Himmelbach, J. Kumlehn, U. Scholz, F. Ordon, and N. Stein. 2014a. Protein Disulfide Isomerase Like 5-1 is a susceptibility factor to plant viruses. Proc Nat Acad Sci USA 111:2104-2109.

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Coordinator's report: *Eceriferum* genes

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Presence of wax coating and its composition is an important feature of the barley plant. It reduces evaporation of water from the plant and helps protect it against pathogens. The waxless *Eceriferum* and glossy mutants affect the presence and type of epicuticular waxes on the different organs. Many different surface wax mutants have been isolated as induced or spontaneous mutants and much research has been done during the last century both genetically and biochemically. All 79 defined loci are published as descriptions in Barley Genetics Newsletter (BGN) 42 and some of the descriptions are updated in this volume. All of them are valid and up-to-date.

One allele of all the 79 gene loci have been backcrossed to a common genetic background the cultivar 'Bowman' by J.D. Franckowiak, USA. They are available as Near Isogenic Lines (NIL) at the Nordic Genetic Resource Centre (NordGen), Sweden, www.nordgen.org and at the Small Grain Germplasm Research Facility (USDA–ARS), Aberdeen, ID 83210, USA, nsgchb@ars-grin.gov. But be aware of that many of tlines are a more advanced backcross derived line incorporated at NordGen than those at the Small Grain Research Facility in Aberdeen. The material in Sweden is well phenotypet and gets regenerated continously.

Li et al. (2015) reported that the *eceriferum-zv* (*cer-zv*) gene which is affecting the surface wax coating and appears absent on the spike, leaf sheath and stem, and leaf blade (wax code - - -) induces drought hypersensitivity in barley. The mutant plants are very semidwarf, and the hull is poorly attached to the seed. Its presence was associated with an increased leaf cuticle permeability to water flux and Toluidine blue molecules and a significant reduction in thickness of leaf cuticle. The *cer-zv* mutant was initially mapped to a 1.7 cM interval in the pericentromeric region of chromosome 4H, but this location has been more narrowed. They used a fine mapping procedure with a progeny equivalent of 7364 gametes to a 0.028 cM interval on the short arm of chromosome 4H. They found a map-based cloning feasable and compared the RNA-seq based transcriptome of the mutant with its wild type and identified 16 genes located in the inspected genomic region. Two of these genes were likely to be functionally related with cutin fomation. The indication is strongly that *cer-zv* is involved in the formation of cutin in the barley cuticle.

Reference:

Li, Ch., G. Chen, J. Ma, K. Mishina, M. Pourkheirandish, Ch. Liu, N. Anwar, P. Zhao, U. Lundqvist, Ch. Nawrath, T.R. Endo, and T. Komatsuda. 2015. Cloning of *cer-zv*, A Novel Gene Responsible for Cutin Formation in Barley Leaf Cuticle. Presented as poster at 'The International Conference on the Status of Plant & Animal Genome Research', San Diego, CA, USA, January 10-14, 2015.

Coordinator's report: Nuclear genes affecting the chloroplast

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Barley chlorophyll mutants have been named *albina*, *xantha*, *viridis*, *chlorina*, *tigrina* and *striata* depending on their colour and colour pattern. In the *albina* mutants the leaves are completely white due to lack of both chlorophyll and carotene pigments. The *xantha* mutants are yellow and produce carotene, but no chlorophyll. The *chlorina* and *viridis* mutants are both pale green, but differ in *chlorina* being viable. The *tigrina* and *striata* mutants are stripped transverse and along the leaves, respectively.

Heterozygous semi-dominant barley magnesium chelatase mutants in the *Xantha-h* locus segregates into yellow homozygous mutants, light green heterozygous mutants and dark green wild type plants. In segregating recessive mutants of the same locus, the heterozygous mutants are dark green and cannot be visually distinguished from wild type leafs. Braumann et al. (2014) characterize the recessive mutations at DNA level in the barley mutants *xantha-h.38*, *-h.56* and *-h.57*. A truncated form of the protein is seen in *xantha-h.38*, whereas no XanH protein is detected in *xantha-h.56* and *-h.57*. With knowledge of the mutations it was possible to genotype individual plants. Spectroscopic analyses revealed that heterozygous mutants show a reduction in chlorophyll content by 14-18% suggesting a slight semi-dominance of *xantha-h.38*, *-h.56* and *-h.57*.

The stock list of barley mutants defective in chlorophyll biosynthesis and chloroplast development is found Barley Genetics Newsletter issue 37 (2007): 37-43 Seeds of most mutants listed can be obtained from Mats Hansson.

New references:

Braumann, I., N. Stein, and M. Hansson. 2014. Reduced chlorophyll biosynthesis in heterozygous barley magnesium chelatase mutants. Plant Physiol. Biochem. 78: 10-14.

Coordinator's report: Early maturity and Praematurum genes

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The demand for early maturity in barley has become an important goal for plant breeding during the last century. Time of flowering has an important impact on yield and has been a key trait in the domestication of crop plants worldwide. Early maturity material has been collected in different geographic regions and climate conditions, today a critical issue in times of global warming. Many different *early maturity* and *Praematurum* mutants are isolated in many different cultivars, and they are stored in Gene banks in several parts of the world. Only in Scandinavia about 1250 different mutants have been isolated, their phenotypes described, analysed genetically and used in plant breeding worldwide. The *Praematurum* mutants are grouped into three categories according to their heading and maturity time with a variation between one and ten days: (1) drastically altered earliness; (2) medium increase of earliness; (3) slightly modified earliness. Long term studies made it possible to identify 10 early maturity (*eam*) and 9 *Praematurum* (*mat*) loci, among them also day-length neutral ones. All identified gene loci are incorporated into a common background, the barley cultivar 'Bowman' by J.D. Franckowiak, USA, and he established Bowman backcross derived lines (Near Isogenic Lines, 'NIL'). All these early maturity lines are well phenotyped, documentated and long-time stored in NordGen, Alnarp, Sweden. They are very important and useful for intensive molecular studies, cloning genes and understanding the barley genome.

Several research programs have been going on with *early maturity* and *Praematurum* genes world-wide. Comadran et al. (2012) reports investigations about genetic consequences of adaptation to new environments and could identify signatures of divergent selection in highly differentiated modern-day spring and winter barleys. In one genetically divergent region they could identify a natural variant of the barley homolog of *Antirrhinum CENTRORADIALIS* (*HvCEN*) as a contributor to successful environmental adaption. The distribution of *HvCEN* alleles in a large collection of wild and landrace accessions indicates that this involved selection and enrichment of preexisting genetic variants rather than the acquisition of mutations after domestication. In their studies they detected that most significant time of flowering QTL was in the centromeric region of chromosome 2H, BIN _07.2. They also included in their studies the *Praematurum-c* (*mat-c*) gene with its 31 available alleles and could verify the location to the centromeric region of chromosome 2H. They called the *mat-c* gene also for *Early maturity 6* (*eam6*), but no allelic tests among the two loci are reported. In earlier Barley Genetic Stock descriptions (BGS 98) it became reported that *eam6* is monofactorial dominant and localized to 2HS, about 13.5 cM proximal from the *vrs1* (six-rowed spike 1) locus, near the *gsh5* (glossy sheath 5) locus based on linkage drag near molecular marker ABC167b in 2H bin 08.

Matyszczak, I. 2014 reports her intensive investigation studies on the *Praematurum-b* (*mat-b*) and *Praematurum–c* (*mat-c*) genes. The earliness in *mat-b* is caused by recessive mutations in the *MAT-b* gene which has been initially mapped using the backcross derived Bowman line BW507. After six backcrosses the line was reported to contain the *mat-b* mutation in one of the two introgressed segments on chromosome 2H defined by 25 SNP markers mapped within 18.02 cM, or on chromosome 4H with 3 SNP markers mapped into a 6.45 cM interval (Drucka et al., 2011). But Matyszczak's analyses demonstrated the mutation in BW 507 to be *mat-c.19*. In genotyping-by-sequencing determined the true chromosomal location of *Mat-b* locus on chromosome 7H, and it is located within a 5.74 cM interval in the telomeric region of chromosome 7HL. Further fine-mapping allowed to slightly reduce the *Mat-b* interval. However, the exact position of *Mat-b* remains unsolved.

The earliness in *mat-c* is caused by recessive mutations in the *MAT-c* gene which has been initially mapped using a backcross derived Bowman line to chromosome 2H. High resolution bi-parental mapping of the mutation narrowed down the target introgression to the 0.27 cM interval on chromosome 2HL. By further analyses Matyszczak was able to characterize *mat-c.19* and seven additional *mat-c* mutant alleles as recessive mutations in a barley ortholog of *Arabidopsis thaliana TERMINAL FLOWER 1* (*AtTFL1*) and *Antirrhinum majus CENTRORADIALIS* (*AmCEN*) gene.

Alqudah et al. (2014) reports investigations on natural variation of time to heading in a world-wide spring barley collection of 218 accessions comprising of 95 d photoperiod-sensitive (*Ppd-H1*) and 123 accessions with reduced photoperiod-sensitivity (*ppd-H1*) to long-day through dissecting pre-anthesis development into four major stages under greenhouse conditions. The *PSEUDO-RESPONSE REGULATOR* (*HvPRR37*) gene, also known as *PHOTOPERIOD RESPONSE LOCUS 1* (*Ppd-H1*), is the central heading time gene regulated in response to long days. They showed that variation at *Ppd-H1* affects heading time of accessions originating from different geographical regions. The gene *HvPRR37* is located on the short arm of chromosome 2H, and they could identify further genes of the heading time pathway, i.e. *Ppd-H2* that response to short days and is located on 1HL. Several other genes play various roles during plant development and photoperiod response. By using polymorphic SNP data from the 9k array they could divide the investigated collection into two groups, based on the presence of a single diagnostic SNP in *HvPRR37*, namely photoperiod-sensitive (*Ppd-H1*) accessions from those with reduced photoperiod sensitivity (*ppd-H1*). Generally their results suggest that the spring barley collection can be divided into two major groups based to photoperiod and reduced photoperiod sensitivity (*Ppd-H1/ppd-H1*) at heading time. The new approach of combining phenotypic dissection of pre-anthesis development with a high-density marker scan provides an opportunity to better understand the genetic base of time to heading in barley.

References:

Alqudah, A.M., R. Sharma, R.K. Pasam, A. Graner, B. Kilian, and Th. Schnurbusch. 2014. Genetic Dissection of Photoperiod Response based on GWAS of Pre-Anthesis Phase Duration in Spring Barley. PLOS ONE 9(11): e113120. doi:10.1371/journal.pone. 0113120. Pp. 1-27.

Comadran, J., B. Kilian, J. Russel, L. Ramsey, N. Stein, M. Ganal, P. Shaw, M. Bayer, W. Thomas, D. Marshall, P. Hedley, A. Tondelli, N. Pecchioni, E. Francia, V. Korzun, A. Walther, and R. Waugh. 2012. Natural variation in a homolog of *Antirrhinum CENTRORADIALIS* contributed to spring growth habit and environmental adaption in cultivated barley. Nat. Genet. 44:1388-1392.

Druka, A., J. Franckowiak, U. Lundqvist, N. Bonar, J. Alexander, K. Houston, S. Radovic, F. Shahinnia, V. Vendramin, M. Morgante, N. Stein, and R. Waugh. 2011. Genetic dissection of barley morphology and development. Plant Physiol. 155:617-627.

Matyszczak, I. 2014. Characterization of early maturity barley mutants *praematurum-a, -b and –c.* PhD. thesis (Aarhus University, Department of Molecular Biology and Genetics, Faculty of Science and Technology, Denmark.

Coordinator's report: Semidwarf genes

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To demonstrate the effectiveness of mapping-by-sequence procedures, Mascher et al. (2014) examined the x-ray induced multi-noded dwarf (*mnd*) mutant (MHOR474) held in the barley genebank at Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. This collection of x-ray induced mutants was established in the 1950s by Friedrich Scholz (Scholz and Lehmann, 1961). The *mnd* mutant showed a 1/3 reduced in plant height when grown in the field, but it had 8 to 9 elongated internodes compared to 4 to 5 for the wild type parent. Mascher et al. (2014) found that in 5HL the deleted regions of contig 49382 overlapped with the two exons of the gene MLOC_64838.2 annotated as 'Cytochrome P450' and named *HvMND*. Analysis of 37 multi-noded mutants from the Nordic Gene Bank (NordGen) identified 30 that had a deletion or a lesion in the *HvMND* gene. Evaluation of two of Bowman backcross-derived (BW) lines by Sanger sequencing of MLOC_64838.2 revealed that BW520 (*mnd4.e*) had a non-synonymous SNP in the coding sequence of and BW522 (*mnd6.6*) had no detectable gene. The recommended locus symbol for this group of allelic mutants is *mnd6* based on prior descriptions of the mutant phenotype by Gustafsson et al. (1969) and its description in the Barley Genetics Newsletter as BGS 633.

Dockter et al. (2014) screened semidwarf barley mutants to identify those that altered expression of brassinosteroid biosynthesis. In silico mapping of brassinosteroid-related genes in combination with sequencing of barley mutant lines, assigned more than 20 historic mutants to three brassinosteroid-biosynthesis genes (BRASSINOSTEROID-6-OXIDASE, CONSTITUTIVE PHOTOMORPHOGENIC DWARF, and DIMINUTO) and one brassinosteroid-signaling gene [BRASSINOSTEROIDINSENSITIVE1 (*HvBRI1*)]. Alternatives to the widely used, but highly temperature-sensitive *uzu1.a* allele of *HvBRI1*, may provide genetic building blocks for breeding strategies with sturdy and climate-tolerant barley cultivars. These mutants were previously grouped as breviaristatum (*ari*), brachytic (*brh*), erectoides (*ert*), and uzu (*uzu*) types.

Alleles at the *uzu1* (*HvBRI1*, brassinosteroid insensitive 1) locus, mapped to 3HL, included: *uzu1.a* (BGS 102), *uzu1.b* (093AR), *uzu1.c* (R710K), *ert-ii.79* (BGS 135), *uzu1.256* (*ari.256*), *uzu1.297* (*ari-o.297*), and *uzu1.301* (*ari-o.301*). Mutants at the *uzu1* locus are characterized as having reduced culm length, short awns, erect leaf blades with undulating margins, elevated levels of castasterone, and insensitivity to exogenously applied *brassinolide*. Only the *uzu1.a*

mutant was demonstrated to exhibit reduced plant height in response to high temperatures (Dockter et al., 2014).

Alleles at *the ert-t* (*HvBRD*, erectoides-t) locus in 2HS, which encodes a brassinosteroid-6-oxidase, include: *ert-t.55* (BGS 566), *ert-t.437* (NGB112953), *ari-u.245* (BGS 678), *brh3.g* (GSHO 1672), *brh3.h* (GSHO 1673), and *brh3.y* (GSHO 1688), *and brh3.i* (GSHO 1674). Mutants at the *ert-t* locus are characterized as having reduced culm length, short awns, and decreased levels of castasterone (Dockter et al., 2014). Identical DNA changes and uncertain selection history indicate that *brh3.g* and *brh3.h* might in fact have the same mutational event.

Alleles at *the brh13* (*HvCPD*, brachytic 13) locus in 5HS, which encodes C-23a-hydroxylase cytochrome P450 90A1 (CYP90A1), include: *brh13.p* (BGS 656) and *brh13.ac* (*brh18.ac*, BGS 659). Mutants at the *brh13* locus are characterized as having reduced culm length, short awns, slightly coiled peduncle, leaf blades with undulating margins, and decreased levels of castasterone (Dockter et al., 2014).

Alleles at *the ari-o* (*HvDIM*, breviaristatum-o) locus in 7HL, which encodes the barley Δ^5-sterol-Δ^{24}-reductase DIMINUTO, include: *ari-o.40* (BGS 556), *brh.af* (FN 46), *brh14.q* (BGS 148), *brh16.v* (BGS 044), *ert-u.56* (BGS 092), and *ert-zd.159* (BGS 093). The six mutants showed a brassinosteroid-deficient phenotype including short culms, short awns, leaf blades with undulating margins, and decreased levels of castasterone. Two mutants, *ari-o.43* (NGB115894) and *ari-o.143* (NGB115953), were demonstrated to be alleles at the *ari-o* locus (Dockter et al., 2014).

Studying backcross-derived lines of Akashinriki and Bowman, Ali et al. (2014) demonstrated that the *uzu1.a* allele at the uzu or semi-brachytic 1 locus provides some resistance to three additional pathogens: the obligate pathogen Barley Stripe Mosaic Virus (BSMV), the necrotrophic net blotch pathogen *Pyrenophora teres* f. *teres*, and the toxigenic hemibiotrophic fungus *Fusarium culmorum* that causes Fusarium head blight (FHB). Both the reduction in plant height and pathogen resistances have been reported to be mediated via the extracellular domains of the leucine rich repeat receptor of the brassinosteroid insensitive 1 (*BRI1*) gene (Santiago et al., 2013). In *uzu1* derivatives, gene expression studies verified that BRI1 transcript levels were high compared to those in the parental line Akashinriki (Ali et al., 2014).

Kuczyńska et al. (2014) reported that the semidwarf 1 (*sdw1*/*denso*) gene had a pleiotropic effect on tillering, plant height, heading or flowering date, and yield. The association between the *sdw1* locus and grain weight per spike and 1000-grain weight was determined to be caused in part by linkage. Their study was based on the 200 single seed descent (SSD) and 60 doubled haploid (DH) lines from a cross between the semidwarf Maresi (two-rowed German cultivar) and Pomo (six-rowed Finnish cultivar). The microsatellite markers (SSR) Bmag0013, Bmag0877, Bmag0306b were used to identify co-segregation at the *sdw1* locus. Lines with the recessive allele at *sdw1* locus were on average about 10 cm shorter than those with the dominant allele.

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Coordinator's report: ear morphology genes

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The research carried out in 2014 on the genetic analysis of the ear mutants of barley has been very intensive.

From one hand new mutants have been morphologically described, and from the other hand molecular characterization and regulation of gene expression have been analyzed. In this report four new ear mutants individuated in the nurseries of the field trials of The "Italian" Barley Genetics Mutant Collection in Fiorenzuola will be shortly described.

In a population of double haploid of the Oregon Wolfe Barley, developed by Pat Hayes, grown at Fiorenzuola, several ear mutants and double mutants have been identified. One has been considered as very interesting because at heading all plants in the small plots showed a complete "**white head**" (Fig. 1). Crosses will be performed to study the genetics of this mutant.

Fig.1- “White head” in OWB (photo Alberici, Pagani)

In a progeny “Leafy-Lemma” few plants showed spikes partially modified, characterized by several spikelets “**lemma-less**” (Fig.2). The remaining spikelets were “Leafy-Lemma”. Is “Lemma-less” a new mutation? A progeny test of single seed will be grown in pots in the glasshouse to confirm the mutation.

Fig. 2 - “Lemma-less” Spikelets (Photo Alberici, Pagani)

By evaluating an F2 population derived from a cross “many glumes on the lateral 1 (*mgl1*) x elongated outer glumes 1 (*eog1*)” few plants showed a small spike with reduced number of internodes and spikelets (fig 3, 4, 5). We have proposed to name this mutant “**reduced number of internode rachis” or “reduced number of spikelets**”. This mutant will be morphologically characterized in detail and used for crosses to perform genetic analysis.

Fig 3, 4, 5: *mgl* (left) x *eog1* (right): in the middle the new mutant "reduced number internode rachis"(Photo Alberici, Pagani)

In a double mutant progeny "deficiens/Hooded" a new mutant has been found, characterized by a portion of the rachis without nodes or spikelets, located in the sub-terminal part of the rachis, and at the top part of the spike two-three final nodes with seeds are present. For this character the mutation has been named "**satellite**" (Fig.6). However this mutant could be regulated by the ***labile*** gene which induces from zero to three spikelets at each node of the rachis. If the mutation will be confirmed, crosses will be performed for further genetic studies.

Fig.6: - “satellite” (or “labile”?) mutant (Photo Alberici, Pagani)

Several papers have been published on the molecular analysis of structure and expression of genes affecting ear morphological components.

Historically the most important trait related to the evolution of barley is the transformation of brittle into non-brittle spike. Throughout the process of barley domestication, the effect of human selection under cultivation resulted in a plant type that produced an ever increasing amount of harvestable grain. Loss of the natural mode of grain dispersal was perhaps the most important single event in this process. At maturity, the spike in wild (i.e. ancestral) barley forms ‘‘constriction grooves’’ and disarticulates at each rachis node, allowing mature grain to disperse freely. This phenotype is referred to as ‘‘brittle rachis.’’ Classical genetic studies have established that a mutation in either of two complementary and tightly linked genes on barley chromosome 3H, *Non-brittle rachis 1 (btr1*) or *Non-brittle rachis 2 (btr2),* converts the brittle rachis into a non-brittle type. Wild-type dominant alleles of both genes are required. Pourkheirandish et al. (2015) identify *Btr1* and *Btr2* genes and elucidate the mechanism underlying disarticulation of the wild-type barley spike. Independent recessive mutations in each of these genes caused cell wall thickening in a highly specific grain ‘‘disarticulation zone,’’ converting the brittle floral axis (the rachis) of the wild-type into a tough, non-brittle form that promoted grain retention. Using comparative DNA sequence information and archaeo-botanical data, the authors demonstrated independent origins of barley domestication. Two ‘‘transition zones’’ were found, where major frequency changes between *btr1*- and *btr2*-types occur: the region between Iran and Afghanistan and the Levant and the southern part of the Mediterranean Sea. Besides these two transition zones, *btr1*-types were found to predominate in India and Ethiopia. While possible that *btr1*- and *btr2*-type barleys may be

better adapted to different eco-climatic zones, an alternative scenario is that their current distribution is a direct result of human migration.

In summary, it appears that the non-brittle rachis evolved from wild barley as a result of anthropogenic selection for mutations in two adjacent complementary dominant genes, the products of which are suggestive of a signal transducing receptor and its protein ligand that likely act in concert to control cell wall thickening in the disarticulation zone of the rachis node. However, the underlying mechanism for this process remains unclear.

Four separate strands of evidence indicate that the *btr1*- and *btr2*-type barleys emerged independently in both time and location. The first centres on the observation that the most closely related sequence haplotypes in the wild species are closer to current cultivated *btr2*-type haplotypes than to *btr1*-type haplotypes; the second is that the archaeological record supports the pre-domestication cultivation of wild barley in the southern Levant occurring earlier. By tracing the evolutionary history of allelic variation in both genes, it can be concluded that spatially and temporally independent selections of germplasm with a non-brittle rachis were made during the domestication of barley by farmers in the southern and northern regions of the Levant, actions that made a major contribution to the emergence of early agrarian societies.

A second mutation affecting the node of rachis is related to the capacity to produce branch or to reduce or enhance the number of spikelets in each node. Spike-branching is of particular importance for enhancing sink capacity and boosting the yield potential of the crop, because in the case of wheat cultivars, current performance is generally thought to be sink-restricted. 'Compositum-Barley' and tetraploid 'Miracle-Wheat' (*T. Turgidum* convar. *compositum* (L.f.) Filat.) display non-canonical spike-branching in which spikelets are replaced by lateral branch-like structures resembling small-sized secondary spikes. As a result of this branch formation 'Miracle-Wheat' produces significantly more grains per spike, leading to higher spike yield isolated. The gene underlying spike-branching in 'Compositum-Barley', i.e. *compositum 2* (*com2*) has been isolated. Moreover, Poursarebani et al (2015) demonstrated that *COM2* is orthologous to the *branched headt* (*bht*) locus regulating spike-branching in tetraploid 'Miracle-Wheat'. Sequence analysis of the *bht* locus in a collection of mutant and wild type tetraploid wheat accessions revealed that a single amino acid substitution in the DNA-binding domain gave rise to the domestication of 'Miracle-Wheat'. mRNA *in situ* hybridization, microarray experiments, and independent qRT-PCR validation analyses revealed that the branch repression pathway in barley is governed through the spike architecture gene *Six-rowed spike 4* regulating *COM2* expression, while *HvIDS1* (barley ortholog of maize *INDETERMINATE SPIKELET 1*) is a putative down-stream target of *COM2*. These findings presented here provide new insights into the genetic basis of spike architecture in *Triticeae*, and have disclosed new targets for genetic manipulations aiming at boosting wheat's yield potential. The gene *com2* underlying spike-branching in barley has been positionally cloned and found that it is orthologous to *bht* that regulates spike-branching in 'Miracle-Wheat' (Poursarebani et al 2015).

The inflorescence of cultivated barley (*Hordeum vulgare* L.) is an indeterminate spike that produces three single flowered spikelets at each rachis internode with one central and two lateral spikelets. Based upon lateral spikelet fertility, barley is classified into two- and six-rowed varieties. Apart from two- and six-rowed barleys, there is another row-type class, which is better known as *labile*-barley (*Hordeum vulgare* L. convar. *labile* (Schiem.) Mansf.) originally described as an irregular row-type of Abyssinian barley. The *labile*-barley displays

a variable number of fertile spikelets at each rachis internode (0–3 fertile spikelets/rachis internode) which is intermediate between that observed in two- or six-rowed type. Youssef et al (2014) report a detailed phenotypic analysis of spikelet fertility in *labile*-barleys in comparison to two- and six-rowed genotypes, using scanning electron microscopy analysis. It has been found that the first visible morphological deviation occurred during the stamen primordium stage, when the authors regularly observed the appearance of arrested central floral primordia in *labile* but not in two- or six-rowed barleys. Two F2 mapping populations have been used to generate whole genome genetic linkage maps and ultimately locate the *lab* locus as a recessive Mendelian trait to a 4.5–5.8 cM interval at approximately 80 cM on chromosome 5HL. The results will help identifying the role of the *lab* gene in relation to other spikelet fertility factors in barley. Possibly due to their high phenotypic row-type plasticity and restricted regional occurrence, *labile*-barleys are genetically at least described among all the naturally occurring row-type variants. Moreover, *labile*-barleys showed another interesting feature whereby some central spikelets also remained reduced only to glumes without any floral meristem development. Molecular genetic results in combination with the examination of detailed lateral and central spikelet development in *labile*- and other row-types may help elucidate the role of the *lab* gene in relation to other floret development and fertility factors in barley.

Another barley mutant, poly-row-and-branched spike (*prbs*) showed altered inflorescence morphology: complete conversion of the rudimentary lateral spikelets in two-rowed barley into fully developed fertile spikelets similar to the six-rowed phenotype, and additional spikelets in the middle of spike. Moreover, branched spikes emerged in progeny from a cross between the mutant and a six-rowed barley cultivar. Morphological observation of the development of immature spikes of the mutant and descendants with branched spikes showed that the *Prbs* gene is involved in spikelet development in the triplemound stage. In mutant *prbs*, new meristems initiated at the flanks of lateral spikelets and middle spikelet meristems were converted to branch meristems, developing branched spikes. These observations suggested that the *Prbs* gene plays a crucial role in spikelet initiation and identity maintenance. The *Prbs* gene may be an important modifier in inflorescence differentiation from a panicle into a spike. The branched spikes emerging in hybrids from a cross between the mutant and six-rowed barley cultivar were not conferred by the gene *vrs1* or *int-c*, which decide spike morphology in six-rowed barley. These results imply that although six-row genes *vrs1* and *int-c* and *prbs* have similar effects on lateral spikelet development, they have different functions in branched spikes. The *Prbs* gene was mapped to chromosome 3H between SSR marker Bmag0023 and marker Cbic60 at a genetic distance of 3.3 and 5.4 centimorgans (cM), respectively (Shang et al 2014).

Despite the economic importance of barley, little has been established relating to the molecular regulation of its pollen development, and to date, no barley male sterile mutants have been characterized. This is partly due to the difficulty of non-destructive staging of floral material, which has now been overcome by the development of vegetative markers linked to key anther stages. Gomez and Wilson (2014) used the Brachypodium genome alongside the rice *PTC1* gene (*AtMS1* orthologue) sequences, to identify conserved regions to amplify equivalent sequences in barley. This approach, combined with RACE-PCR and subsequent functional testing using RNAi lines in barley and complementation analysis in *Arabidopsis*, has led to characterization of the first male sterility gene in barley, *HvMALE STERILITY1* (*HvMS1*). This has demonstrated the conservation of gene function in pollen development between *Arabidopsis* and barley and has provided a valuable tool for the future manipulation of male fertility in barley. The *HvMS1* gene showed highly localized temporal and spatial

expression in the anther tapetum from late tetrad to early microspore release. Barley *HvMS1* overexpression lines showed some similar phenotypes to the *Arabidopsis* MS1 overexpression plants, which showed occasional male sterility and abnormal growth. The barley lines also showed abnormal endothecium expansion and development, with a lack of associated secondary thickening and an increased deposition of cellular materials. The increased expression of *HvMS1* appeared to be causing alteration in cell wall biosynthesis in these anther cell layers. It has been therefore shown that the *HvMS1* gene is critical to pollen development and that when HvMS1 expression is reduced a male sterile phenotype results. This is the first example of a functionally characterized gene in barley that has been specifically linked to pollen development. Controlling crop fertility for hybrid development is a key breeding goal, as increased yield is frequently associated with hybrids. Understanding the molecular process of pollen development is critical to achieving this, and the characterization of such regulatory networks provides the first step in this process.

In barley, the closed floret habit (cleistogamy) is under the control of *cly1*, a gene that operates by inhibiting the development of the lodicule. In non-cleistogamous cultivars, *cly1* mRNA is degraded by miR172-directed cleavage, allowing the lodicules to swell; however, in cultivars carrying the recessive allele *cly1.b*, a single-nucleotide substitution destroys the miR172 target site preventing mRNA cleavage. Barley cv. SV235 is cleistogamous; its *cly1* coding sequence is identical to that of *cly1.b*, but its lodicules do develop, although insufficiently to produce a non-cleistogamous flower. In this cultivar, the downregulation of *cly1* is unrelated to miR172-directed mRNA degradation, but rather is caused by an epiallele that represses transcription. Allelic relationships between known *cly1* alleles were explored by the quantification of lodicule vascularization and an assessment of the response of the spike to the supply of exogenous auxin. The SV235 phenotype can be manipulated by a pre-anthesis application of 2,4-D, a feature that could be of interest in the context of hybrid barley grain production based on cleistogamy (Wang et al. 2015).

Pankin et al. 2014 using Mapping-by-Sequencing identify HvPHYTOCHROME C as a candidate gene for the *early maturity 5* locus modulating the circadian clock and photoperiodic flowering in barley. In this study, it has been described the barley locus *eam5*, which accelerated flowering under LDs and in addition, led to flowering under non-inductive SDs. To fine map the *eam5* mutation, the authors used mapping-by-sequencing of bulked early flowering BC1F2 lines, followed by candidate-gene mapping in BC1F2:3. They demonstrate that fine mapping through exome capture and deep sequencing of a BC1F2 pool was successful, even though the phenotype was quantitative, subitie, and obscured by the segregation of another tightly linked flowering gene. The identification of a mutation in the extremely conserved motif of the PHY GAF domain strongly suggested that *HvPHYC* is the gene underlying the *eam5* locus. Protein modelling revealed that the amino-acid change occurred at a prominent position in the GAF domain at the end of a helix coming to the chromophore pocket, potentially affecting conformational flexibility of the protein successfully applied in model species to map and identify.

The use of Genotyping-By-Sequencing (GBS) on a recombinant inbred line population (GPMx) derived from a cross between the two-rowed barley cultivar ‘Golden Promise’ (*ari-e.GP/Vrs1*) and the six-rowed cultivar ‘Morex’ (*Ari-e/vrs1*) to map plant height has been explored. Liu et al (2014) identified three Quantitative Trait Loci (QTL), the first in a region encompassing the spike architecture gene *Vrs1* on chromosome 2H, the second in an uncharacterized centromeric region on chromosome 3H, and the third in a region of chromosome 5H coinciding with the previously described dwarfing gene *Breviaristatum-e*

(*ari-e*). GBS was an effective and relatively low-cost approach to rapidly construct a genetic map of the GPMx population that was suitable for genetic analysis of row type and height traits, allowing to precisely position *ari-e.GP* on chromosome 5H.

The induction of flowering is a key developmental decision in a plant's life cycle, and its timing is an important adaptive trait for both wild and domesticated plants. The duration of light during the day, known as photoperiod, is one environmental signal used by plants to identify conditions favorable for flowering. Flowering in plants such as wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), pea (*Pisum sativum*), and *Arabidopsis thaliana* is strongly promoted under long-day (LD) conditions, with transcriptional activation of FLOWERING LOCUS T-like genes (FT1 in barley) being a key determinant of the flowering response. During crop domestication, however, breeders have identified plants that display reduced photoperiod sensitivity to assist migration of crops to latitudes where the shorter day lengths would otherwise impede floral induction.

EARLY FLOWERING3 (ELF3) is a circadian clock gene that contributes to photoperiod-dependent flowering in plants, with loss-off-function mutants in barley (*Hordeum vulgare*), legumes, and *Arabidopsis thaliana* flowering early under non-inductive short-day (SD) photoperiods. The barley *elf3* mutant displays increased expression of FLOWERING LOCUS T1 (FT1); however, it remains unclear whether this is the only factor responsible for the early flowering phenotype. Boden et al (2014) show that early flowering and vegetative growth phenotypes of the barley *elf3* mutant are strongly dependent on gibberellin (GA) biosynthesis. Expression of biosynthesis gene, *GA20oxidase2*, and production of the bioactive GA, GA1, were significantly increased in *elf3* leaves under SDs, relative to the wild type. Inhibition of GA biosynthesis suppressed the early flowering of *elf3* under SDs independently of FT1 and was associated with altered expression of floral identity genes at the developing apex. GA is also required for normal flowering of spring barley under inductive photoperiods, with chemical and genetic attenuation of the GA biosynthesis and signaling pathways suppressing inflorescence development under long-day conditions. These findings illustrate that GA is an important floral promoting signal in barley and that ELF3 suppresses flowering under non-inductive photoperiods by blocking GA production and FT1 expression.

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