



**The 12th International
Symposium on Pre-Harvest
Sprouting in Cereals**

JULY 24 - 27 2011

Red Deer, Alberta, Canada

PROCEEDINGS

Don Salmon

Dr. Don Salmon passed away on August 28, 2010. Don was born in Birtle, Manitoba to Harold and Lillian Salmon. Don is survived by his wife, Ferne Gudnason, his mother, Lillian, his brother and sister-in-law, Ray and Sandra Salmon, and their son, Tyson. Don had a deep love of horses that was shared by his late father, his brother and his nephew. Don's breed of choice was the Morgan. He served for many years as the ring master at Morgan horse shows in Alberta and was Zone Director for the Canadian Morgan Horse Association.



Don received his B. Sc. in Agriculture and his Ph.D. in Plant Breeding and Genetics from the University of Manitoba. After graduating, Don was employed by the Saskatchewan Wheat Pool, first as a Wheat Breeder in Zambia, Africa and then in Watrous, Saskatchewan, Canada.

In 1980, "Dr. Don" began his career with the Field Crop Development Centre in Lacombe, Alberta, Canada. His focus was on the development of winter and spring triticale varieties, and along the way this included improving winter wheat. He was instrumental in raising the profile of these crops in Alberta and across Canada. Don was highly regarded by the seed producers of Alberta and other growers of triticale, as he shared his knowledge with them and supported them in their efforts in crop production.

Don was highly respected within the national and international scientific community for his knowledge and efforts to increase utilization of triticale. He mentored many graduate students in his role as an Adjunct Professor with the University of Alberta. He formed strong bonds with breeding programs in Oregon, Mexico, Australia, and others around the world. The germplasm he developed is in use globally and contributes to the provision of a stable food source in many countries.

Don's breeding efforts produced nine varieties of triticale that have been grown across Canada and internationally. The newest of his triticale varieties are highly productive reduced-awn types for livestock feed, livestock forage, and ethanol production. Don worked closely with Dr. Vern Baron, to study the use of winter and spring cereal mixtures for forage use. Together these two researchers developed a production model for annual forage production using spring/winter cereal mixtures to improve the quality of cereal silage and extend the growing season for grazing. They characterized the

carbon balance of the winter cereal within the spring/winter mixture that was fundamental to the fall grazing potential and overwintering of the winter cereal. Currently they were studying the use of cereals for swath grazing.

Don actively participated in the study of pre-harvest sprouting and attending many of the International Pre-Harvest Sprouting Symposiums. His research focused on the dormancy coming from the chaff versus that dormancy coming from the embryo. Don was a member of the organizing committee for the 12th ISPHSC.

Don's contributions to the agriculture industry will not be forgotten. He will be missed by those in the industry and especially by his co-workers with the Field Crop Development Centre for his knowledge, his practicality, his sense of humor and most importantly his friendship.

Preface

On behalf of the Local Organizing Committee and the International Committee for the 12th International Symposium on Pre-Harvest Sprouting in Cereals (12th ISPHSC) I would like to welcome everyone attending the 12th ISPHSC. This is a summary, in abstracts, of the proceedings of the 12th ISPHSC held at the Capri Centre Hotel, Red Deer, Alberta, Canada July 24 – 27, 2011. The purpose of this multidisciplinary meeting is to keep communications among those actively involved in and working in problems related to pre-harvest sprouting. The program for the 12th ISPHSC covers a wide range of topics including farmer perspectives; research including plant breeding, molecular biology, plant and seed physiology and laboratory assay methods. This wide range of topics is a solid platform for participants from across the world representing over 12 countries to discuss about pre-harvest germination, sprouting damage and seed dormancy.

Following the trend of the previous symposia, the 12th ISPHSC takes a relaxed format to encourage more participant interactions and networking. We believe this will encourage a productive exchange of research outcomes and generation of new ideas and solutions to pre-harvest sprouting damage problems. There is also an additional chance for presenters of papers to have their papers published, without page charges, in a Special Issue of *Euphytica*, the International Journal of Plant Breeding. Check the 12th ISPHSC website and follow the links on how to submit your manuscript to *Euphytica*. Proceedings of previous symposia have been widely cited and they form the solid foundation of the current research on pre-harvest sprouting. The 1st ISPHSC was in Sweden in August 1975. The proceedings of the past symposia are in:

1. Cereal Res. Commun. Vol. 4 (2), 1976.
2. Cereal Res. Commun. Vol. 8 (1), 1980.
3. 3rd ISPHSC *In*: J.E. Kruger and D.E. LaBerge, eds. Westview Press, Boulder, CO, USA, 1983.
4. 4th ISPHSC *In*: D.J. Mares, eds. Westview Press, Boulder, CO, USA, 1987.
5. 5th ISPHSC *In*: K. Ringlund, E. Mosleth and D.J. Mares eds. Westview Press, Boulder, CO, USA, 1990.
6. 6th ISPHSC *In*: M.K. Walker-Simmons and J.L. Reid, AACC, MN, USA, 1992.
7. 7th ISPHSC *In*: Kaz. Noda and D.J. Mares, eds. Osaka, Japan, 1995.
8. 8th ISPHSC *In*: D. Weipert, ed. 1998. Detmold, Germany, 1998.
9. 9th ISPHSC *In*: Guest Eds. A.Keith Cowan, *Euphytica* 126: 1-152, 2002.
10. 10th ISPHSC *In*: CD, Norfolk, England June 7-11, 2004.
11. 11th ISPHSC *In*: Guest Eds. R. Benech-Arnold and J. Nyachiro, *Euphytica* 168: 289 - 403, 2009.

The Local Organizing Committee is thankful to the many people that contributed to the success of the symposium. The Alberta Barley Commission and Barley Development Council provided the lead contributions that enabled us to initiate hosting the symposium. The Field Crop Development Centre (FCDC) staff provided support in crucial day-day-planning and logistics of hosting the symposium. In particular, much thanks to Frances (Fran) Teitge and Lori Oatway for their skillful handling of the symposium correspondences, financial matters, website, abstracts, registrations, information, venue booking, hotel accommodation and many more fine details of the symposium.

The Alberta Agriculture and Rural Development, cereal industry organizations and companies with interest in cereals are sponsors of the symposium. We thankfully appreciate the generous financial support of the Symposium sponsors.

For the local organizers,
Joseph Nyachiro

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Organizing Committee

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Notice of next meeting

The 13th International Symposium on Pre-Harvest Sprouting in Cereals is tentatively scheduled for 2014 in _____.

The committee for that meeting will be:

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____
8. _____

Program

Sunday July 24	
1:00 - 5:00 pm	Meetings
4:30 - 5:30 pm	Barley Development Council – Annual Meeting
5:30 - 8:00 pm	Registration
6:00 - 9:00 pm	Welcome Reception and Mixer

Monday July 25	
Session 1. Symposium Opening	
Chair: Dr. Joseph Nyachiro	
7:30 am	Registration / Posters set up 7:30 am & noon
8:15	Welcome and Official Opening: Mayor Morris Flewwelling, City of Red Deer
8:30	R and D support for pre-harvest sprouting and seed dormancy (Jim Helm)
8:55	Breeding for pre-harvest sprouting resistance in Canadian wheat: challenges & successes (Ron DePauw)
9:20	Pre-harvest sprouting and seed dormancy - producer perspective (Bill Chapman)
9:45	Pre-harvest sprouting - an end product quality perspective (Lisa Nemeth)
10:15	Morning coffee/tea break
Session 2. Evaluation and Management of Pre-Harvest Sprouting and Seed Dormancy	
Chair: Dr. Mary-Lou Swift	
10:45 am	Understanding dormancy in cereals and its direct effect on seed quality (Sarah Foster)
11:10	Canadian seed testing laboratory perspective: cereal dormancy and sprouting testing methodology and detection (Holly Gelech)
11:35	Evaluation of an ELISA based rapid assay for the estimation of falling number in Canadian wheat classes (Dave W. Hatcher)
12:00 noon	Lunch
Session 3. Physiology of Pre-Harvest Sprouting, Seed Dormancy and Germination (ISSS Feature)	
Chair: Dr. Pat Juskiw	
1:00 pm	The induction of pre-maturity α -amylase in wheat grains (Kirtkumar R. Kondhare)
1:25	Pre-maturity amylase in wheat (Alison Huttly)
1:50	<i>Vp1</i> expression profiles during kernel development in cereals (Sarah De Laethauwer)
2:15	Investigating the role of ABA signalling in wheat grain dormancy; insights from mutant analysis (Camille M. Steber)
2:40	Afternoon coffee/tea break
Session 4. Genes Signalling Seed Dormancy and Germination	
Chair: Dr. Richard W. Joy	
3:10 pm	Barley <i>ant 28</i> gene encodes an R2R3 MYB domain protein that controls proanthocyanidin accumulation in grain (Eiko Himi)
3:35	A temperature-dependent seed dormancy (TMS) 2 gene acts in the regulation of germination in wheat (Shingo Nakamura)
Field Crop Development Centre Research Farm BBQ	
5:30	Buses leave for FCDC Farm for BBQ (20 minute ride)
9:00	Buses depart FCDC Farm to Capri Centre Hotel

Tuesday July 26

Session 5. Genetics, Breeding and Environment for Pre-Harvest Sprouting and Seed Dormancy

Chair: Dr. Ron DePauw

8:30 am	Germinability during grain development and after-ripening in wheat lines carrying different alleles at dormancy QTL (Daryl J. Mares)
8:55	Analysis of natural variation for seed dormancy and pre-harvest sprouting tolerance in wheat (Francis Ogbonnaya)
9:20	New strategies for discovery loci determining pre-harvest sprouting and dormancy in wheat and barley (Ulrike Lohwasser)
9:45	Environment and genotypic effects on pre-harvest sprouting and seed dormancy in Canadian barley cultivars (Joseph Nyachiro)
10:10	Morning coffee/tea break

Session 6: Genetics, Breeding and Environment for Pre-Harvest Sprouting and Seed Dormancy ... continued

Chair: Dr. Bill Legge

10:40 am	An overview on the effect of climatic conditions on pre-harvest sprouting in South Africa (Annelie Barnard)
11:05	The role of <i>Rht2</i> and 1B/1R in controlling of pre-harvest alpha amylase activity in UK bread wheat (Aidan Farrell)
11:30	Cereal crops pre-harvest sprouting in Iran: previous research and future approaches (Abbass A. Nourinia)
12:00	Lunch

Session 7 : Molecular Biology of Pre-Harvest Sprouting, Seed Dormancy and Germination

Chair: Dr. Jennifer Zantinge

1:00 pm	NCED1 has a principal role in wheat grain dormancy and integrates changes in hormone levels and environmental factors (Jose M. Barrero)
1:25	Toward understanding the gene network underlying wheat pre-harvest sprouting (Lanqin Xia)
1:50	Evaluation of methods of measurement of pre-harvest sprouting resistance in durum wheat (Julio Isidro-Sanchez)
2:15	The manipulation of proanthocyanidin biosynthesis in wheat to investigate the role of grain colour in PHS (Andy Phillips)
2:40	Afternoon coffee/tea break

Session 8: Molecular Biology of Pre-Harvest Sprouting, Seed Dormancy and Germination

Chair: Dr. Ravi Chibbar

3:10pm	Development of molecular markers for sprouting tolerance in spring barley (Jennifer Zantinge)
3:30	Allelic differentiation and downstream gene networks of the pleiotropic gene underlying the association between seed dormancy and red pericarp color in rice (Xing-You Gu)
3:55	Identification of grain dormancy QTL in a barley doubled haploid population derived from non-dormant parents (Lee Hickey)
4:20 - 6:20 pm	Poster Session / Refreshments
	Dinner on your own

Wednesday July 27	
Session 9. Cereal Seed Quality in Relation Pre-Harvest Sprouting and Seed Dormancy	
Chair: Dr. Dave Hatcher	
8:30 am	Falling number – serving the grain community for 50 years (Martin Hallin)
8:55	Study of the effects of pre-harvest sprouting on the storability, malting quality and brewing performance of three Canadian malting barley varieties (Yueshu Li)
9:20	Pre-harvest sprouting and malting quality: which one is more important in barley (Stefan Harasymow)
9:45	Evaluation of alpha amylase accumulation and falling numbers in soft red and soft white wheat adapted to Michigan (Neil Yu)
10:10	Morning coffee/tea break
Session 10. Mapping Factors Related to Pre-Harvest Sprouting, Seed Dormancy and Germination	
Chair: Dr. Anthony Anyia	
10:40 am	Modifying expression of thioredoxin to improve pre-harvest sprouting resistance and other cereal grain properties (Peggy G. Lemaux)
11:05	Association mapping for pre-harvest sprouting tolerance in bread wheat (V. Jaiswal)
11:30	Viviparous-1 gene in the Kazakhstan wheat varieties (Aiman S. Absattarova)
12:00	Lunch / Posters removed
Session 11. Exploring Vivipary, Molecular Markers and Understanding Dormancy in Cereals	
Chair: Dr. Kequan Xi	
1:00 pm	Exploring genetic and epigenetic basis of seed dormancy in cereals (Manjit Singh)
1:25	Barely seed dormancy test: in light or dark (Xue Gong)
1:55	Panel Session: Wrap up of the symposium presentations and discussions
2:55	Closing remarks and vote for next host and venue for the 13 th ISPHSC
3:00	Afternoon coffee/tea break
Farewell Banquet	
5:30 pm	Reception / Cocktails
6:30	Banquet

Sponsors

Platinum Level

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- Brewing and Malting Barley Research Institute
- Canadian Grain Commission
- International Society for Seed Science
- Rahr Malting
- Unity Scientific



Brewing and Malting
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Commission

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des grains



RAHR MALTING



PRESENTATIONS

R & D support for pre-harvest sprouting resistance and seed dormancy in a breeding program – how do we convince the funders that this is worth our time?

Dr. James Helm

Email: James.helm@gov.ab.ca

*Alberta Agriculture & Rural Development, Field Crop Development Centre,
5030 - 50 Street, Lacombe, Alberta, Canada T4L 1W8*

The biggest challenge of getting funding for traits like sprouting resistance or disease resistance is defining the long term economic advantage of these traits. What producers are paid for is tonnes per Hectare delivered. What the market gets paid for is the production of the product being produced. Sprouting resistance and seed dormancy does have a direct effect on both but it is only a small effect except in certain years in certain locations. For a feed barley breeder this is even more difficult as in those years where sprouting is significant most of the sprouted grain goes into the feed market at reduced price. Seed dormancy on the other hand does cause some problems with certain parts of the industry. The malting industry can be the most effected as they want seed that will germinate rapidly and uniformly. But at the same time they don't want it to sprout until they are ready. Over the last 45+ years in the plant breeding business I have found that we have had a few small project funding successes. But to have any long term success you need to make it one of the breeding objectives even if it is a secondary priority. I hope to give you a glimpse of how we have managed this at the Field Crop Development Centre over the last 38 years.

Keyword: Sprouting Resistance, Seed Dormancy, Plant Breeding

Breeding for preharvest sprouting resistance in Canadian wheat: challenges and successes

R.M. DePauw, R.E. Knox, A.K. Singh, D.G. Humphreys, S.L. Fox, and P. Hucl

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RMDP, REK, AKS: Semiarid Prairie Agricultural Research Centre, AAFC, Swift Current, SK, Canada;

GH, SLF: Cereal Research Centre, AAFC, Winnipeg, MB, Canada;

PH: Crop Development Centre, University of Saskatchewan, Saskatoon, SK, Canada

Preharvest sprouting (PHS) in spring wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L. var *durum*) causes significant economic losses due to a reduction in grain yield, grain functionality and viability of seed for planting. Annual estimated losses in Canada are about \$100 million. Genetic resistance to PHS reduces these losses. Development of PHS resistant cultivars is complicated by the effects of factors under genetic control such as spike morphology, seed dormancy and many others, and by environment, and kernel diseases. Resistance to PHS has been a breeding priority since the late 1960s with the development of RL4137 which is the primary source of PHS in the Canada Western Red Spring market class. A white seeded derivative of RL4137 is the primary source of PHS in the Canada Prairie Spring White and Canada Western Hard White Spring wheat market classes. Procedures to select for PHS resistance vary among breeding programs, market classes and by degree of inbreeding. Methods include artificial sprouting of intact spikes, germination tests, natural weathering in field trials, artificial weathering trials, and indirect assessment of sprouting measuring Hagberg falling number. Although many genetic loci have been attributed to preharvest sprouting resistance, at this time application of molecular markers is limited due to the complex inheritance of the trait. In Canada cultivars are characterized for their relative level of PHS resistance and the information is made available to producers.

Economic impact of pre-harvest sprouting, seed dormancy and germination from a farmer perspective

Bill Chapman

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Agriculture and Rural Development, Box 4560, Barrhead, Alberta T7N 1A4

Farmers are often affected by weather conditions that can have serious implications on their income and struggles to produce and harvest their crops. We experienced this combination of cold and dry weather which contributed to a very late harvest in 2010, significantly lowering grain quality. Local areas showed grade reductions to feed with grade losses due to bleaching, and chitting or pre-harvest sprouting as well as seed viability problems of reduced germination. All top grades of cereal especially are based on dry grain with few or any slight problems which directly influences farm returns and net income for farmers. Most farmers in central and northern Alberta have added various types of equipment to reduce the risk and problems associated with pre-harvest problems. Approximately 40% of the farmers have heated air dryers, another 20 to 35% have added aeration fans to their bins with another 10% have added supplemental heat to these aeration fans to dry and condition the grain for on-farm grain storage in central and northern Alberta, with less in the south. Farmers can seed many acres with new large air drills but the whole year and crop income are directly influenced by the farmer's ability to harvest the crop with the highest quality possible. Last year a number of farmers north of Edmonton were able to get # 1 HRS wheat due to the use of straight combining and/or swathing then combining wheat before it lost grade due to wet weather, frost, and sprouting. Each harvest experienced by farmers has very distinct regional differences and weather patterns that can strain or improve crop income as it relates to crop especially cereal grades harvested. A good example of this is the better quality malting barleys can sprout in the field if the regional area receives rainfall at physical maturity and the cold wet weather persists for more than a few days causing chitting or sprouting problems reduce any chance of malting grades for barley. Seed dormancy can help reduce this problem if the rainfall is limited but too much dormancy will significantly impact the malting process delaying germination in steep tanks and starch conversion times.

Pre-harvest sprouting - an end product quality perspective

Lisa Nemeth

The Canadian Wheat Board
Email: Lisa.Nemeth@cwbc.ca

423 Main Street, P.O. Box 816, Winnipeg, Manitoba, Canada R3C 2P5
Phone: (204) 983-0135 Fax: (204) 984-1699

The impact of pre-harvest sprouting on end-products produced from wheat and durum depends on the amount of enzymes present and the breakdown of the kernel. Damage from pre-harvest sprouting can affect flour extraction but is most commonly associated with the starch degrading enzyme α -amylase. This presentation will examine the effects of α -amylase on the quality of several different end products and explain why some end products can tolerate higher levels of sprouting and still maintain a good quality. It will also illustrate how millers can blend several sources of wheat, with various levels of sprout damage, to achieve an acceptable quality but that this blending requires careful calculation.

Understanding dormancy in cereals and its direct effect on seed quality

Sarah Foster

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2020 Seed Labs, Suite 201, 509-11th Avenue, Nisku, Alberta, CANADA T9E 7N5

Seed dormancy in cereals can significantly affect seed quality and ultimately impact the economic returns to the seed grower. Environmental and chemical factors during seed maturation can induce dormancy and depending upon the situation, germination and vigour may be seriously affected. In many cases this can cause reduced emergence the following spring. The responsibility of the seed laboratory is to determine the true germination and vigour value by determining the level of dormancy. If seed dormancy is suspected, several methods such as temperature regime and chemical processes that are known to break the dormancy are routinely used during the testing period. It is important to break dormancy in the laboratory because it masks other issues such as chemical damage, frost and diseases. Seed vigour is also affected by dormancy and consequently abnormally low seed vigour test results are often observed. Seed dormancy is usually broken during seed storage over the winter at sub zero temperatures, however with extreme dormancy it can be observed at seeding time in the spring. Reasons for dormancy, types of dormancy and seed laboratory analyses for the determination of seed quality will be discussed.

Canadian seed testing laboratory perspective: cereal dormancy and sprouting testing methodology and detection

Holly Gelech

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Tens of thousands of cereal seed samples are tested for germination by accredited seed analysts each year using CFIA's Methods and Procedures for Testing Seed. The adverse growing season and poor harvest conditions in 2010 resulted in higher prevalence of dormancy compared to prior years. The key indicator of dormancy in the germination test is presence of fresh seeds, which are viable seeds that have failed to sprout, therefore reducing the germination result. During the 2010/2011 testing season, 7.2% of the wheat samples tested at BioVision Seed Labs exhibited a 5% or greater fresh seed percent in the germination test. Similar results were reported in oat and barley seed, with 5.75% and 2.2% respectively of the samples tested demonstrating a 5% or greater fresh seed percent. Quite contrary, the 2009 cereal seed crop was not impacted greatly by dormancy. Canadian accredited seed laboratories employ various techniques to break dormancy including pre-chilling procedures and media additives (potassium nitrate and gibberellic acid). Sprouting is not a reportable characteristic in the germination test, but structural symptoms were observed at elevated levels in the 2010/2011 testing season. In addition to visual assessment, various commercialized tests benchmark pre-sprouting in wheat and barley, including Hagberg Falling Number and Rapid Visco Analysis.

Evaluation of an ELISA based rapid assay for the estimation of falling number in Canadian wheat classes

D.W. Hatcher

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Currently the Canadian wheat grading system evaluates and quantifies sprout damage in wheat on the basis of a subjective, visual assessment of 25 g of wheat. While quantifying the amount of sprouted and severely sprouted wheat has been satisfactory in the past, it is not an ideal means of ensuring contractually obligated quality on the basis of Falling Number. The ELISA based ReadRite[®] system by Bayer CropScience utilizes a representative 0.625g of ground wheat, derived from 300 g of wheat kernels, extracted for 40 s in 8 ml of solution to determine both the alpha amylase and corresponding estimated Hagberg Falling Number of the wheat sample. The equipment required has a minimal bench footprint, comes with its own calibration cassette for daily quality control and the assay is designed for use by a non-technically trained individuals. The ReadRite[®] system was evaluated at multiple sites (4) across the country by assaying representative sub-samples of both Canada Western Red Spring (CWRS) and Canada Western Amber Durum (CWAD) wheat samples in the fall of 2010. Initial studies based on 39 CWRS samples yielded an $r^2=0.94$ with a SEP=18.6 s between the ReadRite[®] estimated Falling Number value and that of the average Hagberg Falling Number determined at 4 different study sites. Analyses of 37 CWAD samples resulted in $r^2=0.97$ and a SEP=16.4 s. Analyses of the traditional Hagberg Falling Number test on the same ground material as used in the ReadRite[®] analyses showed almost an identical range in falling number across the various sites for each sample highlighting the usefulness of the ReadRite[®] test. Currently (2011) a much larger study is underway based on 45 samples of both CWRS and CWAD being analyzed at 15 different sites across the country, including grain companies and private analytical laboratories. The results of this recent large scale study are presented.

The role of abscisic acid and gibberellins during pre-maturity α -amylase formation in wheat grains

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A cold shock during a 'window of sensitivity'; i.e. 26-30 days after anthesis (DAA); induces pre-maturity α -amylase (PMA) in the later stages of grain filling in susceptible wheat (*Triticum aestivum*, L) varieties. During germination, abscisic acid (ABA) inhibits and gibberellin (GA) stimulates α -amylase formation in the embryo and aleurone. The effects of (*in situ* and *in vitro*) applied ABA and gibberellic acid (GA₃) on the occurrence of PMA was investigated in developing wheat grown in glasshouse experiments under control or cold shocked conditions, using UK winter wheat varieties; 'Spark' (PMA resistant) and 'Rialto' (PMA susceptible).

In the *in situ* experiments, the cold shock (12 °C) was given at 26 DAA by transferring plants to an air-conditioned glasshouse for 8 days. 10 μ l of hormones [ABA (100 μ M) and GA₃ (50 μ M)] were applied *in situ* to intact, developing grains in three doses within the 'window of sensitivity'. The α -amylase activity was measured at 60 DAA from distal half-grains using the modified Phadebas assay. The cold shock and GA₃ treatments as their own and cold-shock plus GA₃ treatment resulted in PMA in both varieties. The effects were more pronounced in 'Rialto' than 'Spark'. ABA treatments had no effect on PMA formation in control/cold shocked plants in either variety.

In the *in vitro* experiments, samples from control/cold shocked plants were harvested at three time points; i.e. 31 DAA, 36 DAA and 40 DAA. Distal half-grains obtained were incubated in hormone solutions (*in vitro*) at 25 °C in the dark for 72 h, and PMA produced was analysed by the Megazyme assay. At 31 DAA, applied GA₃ did not produce any effect on PMA in either variety. At 36 DAA, the cold shock and GA₃ treatments as their own and cold-shock plus GA₃ treatment induced PMA in both varieties. At 40 DAA, the cold shock greatly increased PMA in response to applied GA₃ in both varieties. The cold shock had no effect on the ABA-responsiveness in developing grains and therefore, applied ABA did not produce any effect on PMA formation for any of the three time points in either variety. Thus, it appears that GA response/sensitivity is a major factor during PMA induction by the cold shock whereas the ABA response/sensitivity seems to be of less importance.

Abbreviations: PMA, pre-maturity α -amylase; ABA, abscisic acid; GA, gibberellin; GA₃, gibberellic acid; DAA, days after anthesis.

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Pre-maturity amylase in wheat

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Pre-maturity amylase (PMA) is a significant contributor to low Hagberg in wheat. It is distinct from PHS in that α -amylase accumulates during grain development in the absence of sprouting. Within a larger collaboration between UK wheat breeders and researchers covering all aspects of HFN, we have been studying the physiology and molecular biology of PMA. This includes work on the induction of PMA by cold temperature and by manipulation of hormone levels, transcriptome analysis of grain during induction and determination of the location of the α -amylase produced within individual grains. Transgenic reporter lines with GFP driven by different α -amylase promoters have also been generated. Differences in the pattern of amylase expression observed in constitutive and inducible PMA suggest that more than one syndrome is involved.

Vp1 expression profiles during kernel development in cereals

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During the last decades many researchers investigated the physiological and genetic background of dormancy and, in correlation with it, pre-harvest sprouting (PHS). Special attention has often been paid to genetic factors which may explain and predict PHS sensitive behaviour. One of these genetic factors is the *Vp1* gene which is involved in embryo development and maturation as well as in dormancy establishment. In this study, the *Vp1* gene expression during kernel development was determined in wheat, triticale and rye in order to identify its possible use to select for PHS tolerant varieties in cereal breeding programs. Plants of known PHS tolerant and PHS sensitive varieties were grown under controlled conditions from flowering until harvest ripeness. Meanwhile, kernels were regularly harvested for RNA extraction and cDNA synthesis. Normalized and calibrated relative expression levels of *Vp1* were hence obtained in a real-time RT-PCR assay. During kernel development, these *Vp1* expression levels generally show a typical peak during the soft dough stage and the beginning of the hard dough stage, after which they decrease and remain low until harvest maturity, confirming earlier results. Furthermore, differences in these *Vp1* expression levels could be observed between the PHS sensitive and PHS tolerant varieties of wheat, with the PHS tolerant variety showing higher levels of relative *Vp1* expression compared to the PHS sensitive variety. In triticale, however, this observation was only seen once and could not be confirmed in further experiments. It seems that the *Vp1* gene in triticale behaves more in a similar way as in rye, in which no specific trends could be observed.

Investigating the role of ABA signaling in wheat grain dormancy; insights from mutant analysis

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The decision to germinate is controlled by two opposing hormone signaling pathways; ABA triggers dormancy whereas GA stimulates seed germination. Understanding the balance between these signaling pathways is critical to breeding for pre-harvest sprouting tolerance without interfering with efficient germination and crop establishment. As a first step to elucidating this hormonal regulation in wheat, we have isolated mutants with increased and decreased sensitivity to ABA during seed (caryopsis) germination. In wheat, ABA response in seed germination is dependent on dormancy status. Fully dormant wheat seeds fail to germinate; partially dormant wheat grain germinate slowly and fail to germinate on ABA; fully after-ripened wheat seeds germinate well even in the presence of ABA. ABA hypersensitive lines were isolated by screening mutagenized grain after-ripened for 6 months to one year for failed germination on ABA. ABA insensitive mutants were isolated in the hard red spring Scarlet based on the ability to germinate on ABA prior to after-ripening.

Red-grained wheat often has more dormancy than white wheat. In red-grained wheat, we failed to identify mutants that could respond to ABA regardless of how long the seeds were after-ripened. Such ABA hypersensitive mutants could only be scored when the seeds were partly dormant. This phenotypic window made genetic analysis challenging. Two ABA hypersensitive mutants in red Chinese spring called Wheat ABA-responsive mutants, *Warm1* and *Warm4*, show increased seed dormancy and increased sensitivity to ABA during germination of partly dormant grain. Whereas *Warm1* is seed specific, *Warm4* shows decreased leaf transpiration indicative of increased vegetative ABA sensitivity. In contrast to ABA hypersensitive red wheats, mutants isolated in the white-grained background 'Zak' show a persistent increase in ABA sensitivity. One of these lines, *ZakERA0* (*Enhanced Response to ABA-0*) is seed-specific, and shows increased ABA sensitivity compared to wild type even once after-ripened for three years. *ZakERA0* is able to germinate well in the absence of ABA after only one to two months of after-ripening. Thus, this semi-dominant gene shows promise for reducing pre-harvest sprouting of white cultivars without compromising emergence.

Barley *ant 28* gene encodes an R2R3 MYB domain protein that controls proanthocyanidin accumulation in grain

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The red grain color of wheat has been associated with the development of grain dormancy. Also, it affects the brightness of wheat flour due to contamination of red pigment in the milling process. Red pigments in the grain coat are proanthocyanidins (PAs) consisting of flavan 3-ol units (e.g. (+)-catechin and (-)-epicatechin). PAs and anthocyanin are synthesized through the common flavonoid biosynthesis pathway. The genes involved in the pathway (*CHS*, *CHI*, *F3H*, and *DFR*) in wheat were isolated and it was found that, in comparison, an expression of these genes was almost completely suppressed in the developing grains of white-grained wheats to red-grained wheats.

Wheat grain color is controlled by the *R-1* genes located on the long arm of chromosome 3A, 3B, and 3D. Each *R-1* gene (*R-A1*, *R-B1*, and *R-D1*) is dominant and mono-gene inherited. It was previously reported that genes encoding R2R3 Myb domain protein (*Tamyb10* genes), isolated from wheat, might be *R-1* genes. However, it is still unknown how *R-1* genes control both grain color and dormancy, especially sensitivity of the embryo to abscisic acid (ABA). Since wheat is a hexaploid, it is difficult to investigate wheat genetics and/or physiology.

In Arabidopsis, most mutants impaired in flavonoid accumulation have been identified from screenings for altered seed pigmentation which result in *transparent testa* (*tt*) mutants. Seventeen genes have been identified to date at the molecular level encoding structural proteins, regulatory proteins, and proteins that are probably involved in flavonoid compartmentation. In barley, over 600 mutants that are deficient in PA accumulation have been characterized because PAs have a significant effect in determining the quality of beer. These mutants belong to more than 20 *ant* loci, but only one *ant* locus was identified at the molecular level (*ant18* gene is the structural gene for DFR).

In this study, we revealed that a gene encoding R2R3 Myb domain protein isolated from barley is orthologous to *Tamyb10* genes. Moreover, it is the causal gene for barley *ant28* with proanthocyanidin-free grains. This is a first step in the molecular investigation of the relationship between grain color and dormancy with barley *ant* mutants.

A temperature-dependent seed dormancy (*TMS*) 2 gene acts in the regulation of germination in wheat

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Seed dormancy is an adaptive mechanism and an important agronomic trait. Temperature during seed development strongly affects seed dormancy in wheat with lower temperatures producing higher levels of seed dormancy. To identify genes important for seed dormancy, we analyzed gene expression in embryos from mature seeds grown at lower (13°C) and higher (25°C) temperatures using a wheat microarray. We found that a gene designated as temperature-dependent seed dormancy (*TMS*)2 was up-regulated in dormant seeds grown at the lower temperature after physiological maturity. By *in situ* hybridization analysis, *TMS2* was found to be exclusively expressed in the scutellum. Mapping analysis showed that *TMS2* on chromosome 3A (*TMS2-3A*) co-localized with the seed dormancy quantitative trait locus (QTL) *QPhs.ocs-3A.1* which was detected using RILs derived from a cross between dormant cultivar Zenkougikomugi (Zen) and less-dormant cultivar Chinese Spring (CS) (Mori et al. 2005). *TMS2-3A* expression levels in Zen were higher after physiological maturity; this may be caused by a single nucleotide polymorphism found in the promoter region. In a complementation analysis, high levels of *TaMFT* expression were correlated with a low germination index in T₁ seeds. Furthermore, precocious germination of isolated immature embryos was suppressed by transient introduction of *TMS2* driven by the maize ubiquitin promoter. Taken together these results suggest that *TMS2* plays an important role in the regulation of germination in wheat.

Germinability during grain development and after-ripening in wheat lines carrying different alleles at dormancy QTL

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Grain dormancy in many white- and red-grained wheat cultivars is associated with a major QTL on chromosome 4A and from 1 to 3 additional QTL depending on the genotype. Whilst the 4A QTL has been associated with embryo sensitivity to ABA and the 3B QTL with changes in the flavonoid content of the seed coat, these require validation and mechanisms associated with other dormancy QTL are unknown. Genotypes containing single dormancy QTL or combinations of QTL were isolated from doubled haploid populations. Dormancy phenotype, measured as a germination index, and ABA sensitivity were determined at intervals during grain development and after-ripening whilst phenotype at harvest-ripeness was also compared at 2 ripening temperature regimes. Genotypes with different combinations of QTL were characterized by consistent differences in dormancy phenotype at harvest-ripeness. These differences appeared to reflect changes in the length of lag phase that preceded the onset of germinability during grain development since the subsequent rate of dormancy loss that followed the lag phase appeared to be similar in all genotypes. ABA sensitivity changed in parallel with dormancy/germinability of intact grains and did not appear to be determined by a particular QTL. Pyramiding dormancy QTL appeared to progressively extend the lag phase for the onset of germinability.

Analysis of natural variation for seed dormancy and preharvest sprouting tolerance in wheat

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Preharvest sprouting (PHS) is one of the most important factors affecting wheat quality in many wheat producing areas especially in environments characterized by late season rainfall during grain maturation and high humidity prior to harvest. In such environments, the incorporation of sufficient levels of PHS tolerance is necessary to minimize losses associated with Preharvest sprouting damage often results in down-grading of premium milling quality wheat to feed quality, which results in seriously reduced pay-outs to farmers. Preharvest sprouting is a complex trait that is affected by many environmental and genetic cues. To identify loci that determine natural variation for seed dormancy and PHS tolerance in wheat, we carried out comprehensive quantitative trait loci (QTL) analyses of two BC₁F₇ synthetic backcross (SBLs) populations. These populations were derived from crosses involving Syn36 (synthetic hexaploid with moderate seed dormancy) and Syn37 (synthetic hexaploid with high seed dormancy) and Janz (Australian prime hard wheat cultivar, PHS-susceptible) respectively and a set of F₈ recombinant inbred lines (RILs) population derived from the cross between “CN19055” (PHS-resistant) with locally adapted Australian cultivar “Annuello” (PHS-susceptible). Assessment for seed dormancy was based on germination index (GI7) while PHS tolerance was based on whole head assay (sprouting index, SI) and visibly sprouted seeds (VI), the latter two following artificial weathering in a controlled environment chamber. Genomic regions on chromosomes 1D, 2D, 3D, 4A, 5D and 6D were found to have significant impact on seed dormancy and PHS tolerance in wheat. We report for the first time identification of new loci on chromosomes 1D and 2D that control PHST. Coincident QTLs were identified for GI7, SI and VI on chromosomes 4A, 3D and 5D. Molecular markers associated with these traits explained 9 to 43% for GI7, 7 to 13% for SI and 13 to 45% for VI depending on the population. QTL associated with red grain color of *Ae. tauschii* was also separated from seed dormancy to develop white PHS-tolerant wheat germplasm. These results confirmed the role of major loci including 4A and 3D that had previously been reported which that confer seed dormancy and PHS tolerance in wheat. This indicated that the QTLs found in this study are reliable, thus it would be worthwhile to target these conserved loci including the novel loci on 1D and 2D for further molecular characterization, cloning and use in breeding.

New strategies for discovery loci determining pre-harvest sprouting and dormancy in wheat and barley

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Five different populations were grown under field and greenhouse conditions in Gatersleben, Germany, in order to determine loci for pre-harvest sprouting (PHS) and dormancy in wheat and barley. For wheat (*Triticum aestivum* L.) a set of 114 recombinant inbred lines (RILs) of the ITMI population (International Triticeae Mapping Initiative) and 85 *Triticum aestivum* cv. "Chinese Spring"-*Aegilops tauschii* D-genome introgression lines developed in Gatersleben were used to perform a classical QTL (quantitative trait loci) analysis. In addition, a population based on 183 accessions of the German genebank was studied for an association mapping approach. Diversity array technology (DART) profiling was performed by Triticarte Pty. Ltd (Canberra, Australia; <http://www.triticarte.com.au/>) which results in more than 2,000 informative markers. Both models, general linear model (GLM) and mixed linear model (MLM), were calculated. For barley (*Hordeum vulgare* L.) two populations, 94 double haploid lines (DH) of the OWB-population (Oregon Wolfe Barley) and 150 DH lines of Steptoe/Morex (SxM) were investigated. Beside the classical QTL analysis an integrated genetic map of barley based on markers developed from 1,032 expressed sequence tags (ESTs) was used to link PHS and dormancy to the function of ESTs.

Results from ITMI show major QTLs on chromosomes 3AL and 4AL for dormancy and PHS. For the D-genome introgression lines a major QTL on 6DL could be found. The association mapping population explains more than 25 marker trait associations with again important regions on chromosomes 3AL and 4AL. Other detected deletion mapped DART markers for sprouting on chromosome 1AS and 1DL correlate with disease resistance genes. For dormancy an association with a salt-responsive gene could be found on chromosome 3AL. In barley interesting regions are on chromosomes 5H and 7H. One of the detected functions is an iron/ascorbate dependent oxireductase which is known as a stress response gene. Further evaluations will show possible homologous regions of wheat and barley. And the results between bi-parental and multi-parental populations will be compared.

Environment and genotypic effects on pre-harvest sprouting and seed dormancy in Canadian barley cultivars

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Cereal seed dormancy and pre-harvest sprouting are interrelated traits influenced by environment. The objective of this study was to determine if there are any genotypic differences in spike sprouting and seed dormancy among 46 Canadian barley varieties (genotypes) and find if there is any association between spike sprouting and whole seed dormancy. The genotypes were grown for three years at two locations near Lacombe and Olds, AB in a 2-replicate randomized block design. Three intact spikes, mainly from the primary tillers, were evaluated for resistance to sprouting resistance in a rain simulator at 18 °C. Sprouting was rated visually on a 1-5 scale (1= no visible sprouting, 5= 100% sprouted) and ratings were converted to spike sprouting indices (SSI) that took into account the promptness of spike sprouting. The genotypes were designated as resistant (R) to sprouting if they had a SSI range of 3.0 to 4.0; moderately resistant if 4.1 to 5.0; susceptible if 5.1 to 6.0; and very susceptible (VS) if >6.0. Also whole seeds for each line were tested for seed dormancy based on a weighted germination index (WGI). Continuous variations were observed both in the SSI and WGI among genotypes. There were genotypic differences for spike sprouting ranging from 3.1 (R) to 7.3 (VS). For the SSI, the hulless barley varieties ranged from 3.5 to 5.9, the 2-rowed ranged from 4.7 to 6.5 and the 6-rowed ranged from 3.8 to 6.5. There were wide variations in WGI ranging between 0 (no seed germination) and 1 (100% seed germination). For the WGI, the hulless barley varieties ranged from 0.2 to 1, the 2-rowed ranged from 0.1 to 0.9 and the 6-rowed ranged from 0 to 0.8. The cultivars Vivar and Xena (feed barley) consistently appeared to have good resistance to spike sprouting. The correlation between the WGI and SSI ($r = 0.37$), although significant ($P > 0.01$), appear to be weak. The lack of a strong correlation between spike sprouting index and weighted germination index indicates that these parameters could be independent of each other. Our results demonstrate that there is dormancy imposed by the hull and seed per se and the two factors could be acting together or exclusively independent of each other. Environment (E), genotypes (G) and G x E contributed significant variations to pre-harvest sprouting and seed dormancy of the tested varieties.

An overview on the effect of climatic conditions on preharvest sprouting in South Africa

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In South Africa, rainfall occurs in most seasons during the grain harvesting period. Preharvest sprouting (PHS) is a risk factor in wheat production in South Africa as even mild sprouting affects the quality of the grain. During recent years high levels of PHS in wheat was experienced in parts of South Africa, especially in the summer rainfall area. The extent of PHS is hard to predict. In this study we investigated the effect of temperature and rainfall on PHS values of selected cultivars by using the meteorological data from planting to harvest. To establish a quantitative relationship between PHS and different climatic characteristics, wheat cultivars were planted in three regions representative of the winter wheat growing conditions of South Africa over four years. Climatic characteristics during six environmental periods were investigated, namely planting to harvest, anthesis to harvest, grain filling, 14 days prior to physiological maturity and 10 and 20 days prior to harvest respectively. These data sets were correlated with PHS resistance determined in a rain simulator to determine if climate during various stages of grain development had an effect on the expression of dormancy and subsequent PHS. Principal component analysis (PCA) on mean PHS values identified three distinct groupings of cultivars, ranging from PHS susceptible to PHS resistant. A fairly strong positive correlation ($r=0.715$, $P=0.008$) was found between PHS and minimum temperature during grain filling, which implied that lower minimum temperatures during the grain filling period, coupled with higher maximum temperatures during the later stages of grain development, could be associated with higher PHS resistance in certain cultivars. Large variations in PHS values were also observed between the various cultivars, indicating that certain cultivars, such as Caledon, Gariiep, Limpopo, Matlabas, PAN 3118, PAN 3120, PAN 3377 and SST 334 are more sensitive to environmental effects than others and that the variation in cultivar PHS is not consistent across sites and years. This study also showed that individual cultivars reacted differently with regard to climatic characteristics during the various stages of grain development. While the PHS susceptible and PHS tolerant cultivars were unaffected by climatic changes, the intermediate group reacted differently. In only one cultivar, rainfall during the period before harvest had an effect on the PHS expression of the cultivar.

The role of *Rht2* and 1B/1R in controlling pre-maturity alpha amylase activity in UK bread wheat

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The occurrence of pre-maturity alpha-amylase (PMA) was investigated in two double haploid wheat populations segregating for *Rht-D1b* (*Rht2*) and the 1B/1R translocation. Genotypes were assessed in the field and in controlled environments with a cold-shock applied to induce PMA. Results from field-grown Spark x Rialto genotypes suggest both *rht2* and 1B/1R increase the expression of PMA with their influence being independent and additive. In Option x Potent genotypes, fixed for *Rht2*, the 1B/1R effect was similar to that seen in the equivalent Spark x Rialto genotypes. Under controlled environment conditions the 1B/1R effect was more pronounced than in the field in both control and cold-shock treated plants. No interaction was found between 1B/1R and the cold-shock treatment, with 1B/1R present in the majority of genotypes expressing PMA under control and cold-shock conditions. Overall, the results suggest the 1B/1R translocation increases the expression of alpha-amylase in PMA prone germplasm independently to effects of *Rht2*.

The cereals crops pre-harvest sprouting in Iran: previous research and future approaches

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Damages of pre-harvest sprouting (PHS) on cereals in moist zones are relatively common and occur 3 or 4 times per 10 years. Pre-harvest sprouting negatively affects the chain of production from the field to baking operations. Genetics and weather conditions play a large role in the PHS resistance of cereals. In Iran, pre-harvest sprouting occurs during harvest season because of rainfall and high humidity in May in north regions for example raining in June 1993 caused to pre-harvest sprouting (PHS) in more than 50000ha wheat farm. This phenomenon make decrease yield and grain quality and results economical losses to cereal growers, consumers and also government. Because of high genetic diversity, this problem can be studied in breeding research. After 1995 result of research in agronomy plant such as wheat and rice help to introduce pre-harvest sprouting (PHS) tolerant genotypes. Research has showed that the major factor in cereal pre-harvest sprouting in Iran is late planting and using late maturity cultivars. In addition to, different weather conditions in different zones of Iran had different effects on PHS. The aim of this study is to review previous research on cereals PHS in Iran and to find strategies to overcome this phenomenon on the cereal farms of Iran.

Keywords: *Cereals, Iran, pre-harvest sprouting*

***NCED1* has a principal role in wheat grain dormancy and integrates changes in hormone levels and environmental factors**

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It is well known that changes in plant hormone responses play an important role in loss of grain dormancy by after-ripening in cereals, but little is known about the after-ripening process that triggers those changes. Loss of dormancy by after-ripening is strongly associated with a change in abscisic acid (ABA) sensitivity and catabolism in the cereal embryo, and the inability of dormant grains to germinate is associated with cereal embryos retaining a high ABA content together with high sensitivity to the hormone during imbibition. As part of our interest in understanding how after-ripening alters dormancy we have been investigating the role of light and hormones in regulating ABA content and sensitivity in dormant wheat and barley grains. Our results show that blue light is a strong promoter of dormancy and that jasmonate (JA) releases grain dormancy in wheat. Very interestingly, we have found that both blue light and JA target ABA biosynthesis by the activation or the repression of the same target gene, *NCED1*. We propose a central role for *NCED1* in integrating changes in hormone levels and environmental factors. We are now investigating how *NCED1* is regulated by *NCED1*promoter:GUS reporter constructs in transgenic barley plants.

Toward understanding the gene network underlying wheat pre-harvest sprouting

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Pre-harvest sprouting (PHS) of kernels while they are still in the ear, occurs usually in response to damp conditions and is due to an early break of seed dormancy. PHS results in lower yield and a reduction in end-product quality, and could cause great economic losses to farmers. It occurs frequently in many parts of the world including China and poses great threat to wheat production in these countries. Therefore, understanding the mechanism underlying wheat PHS therefore becomes a top issue in wheat genetic improvement.

Viviparous1 (Vp1), a major regulator of late embryo development in maize, could promote embryo maturation and repress germination. Orthologues of *Vp1* have been cloned and mapped to the long arms of the group 3 chromosomes. Alternative splicing of the pre-mRNA of three *Vp1* homologues in common wheat have been implicated in PHS susceptibility and transgenic wheat seeds expressing the *AfVp1* cDNA showed increased dormancy and tolerance to PHS. Furthermore, red grain color of wheat is also associated with the development of grain dormancy, but it is not sufficient to guarantee dormancy. *Myb/c1(R)* is transcriptional activator of the flavonoid synthesis genes. *VP1* can interact with the *Sph* cis element in the promoter region of *R* gene and regulate its expression, whereas the *Myb/c1* transcription factor could bind to the *P* element in the promoter region of the *DFR* (dihydroflavonol-4-reductase) genes which are located on wheat chromosomes 3A, 3B and 3D and control the anthocyanin biosynthesis pathway to generate grain color. And the genes involved in flavonoid biosynthesis, including *DFR*, could respond to both *VP1* and *ABA*.

We isolated and characterized several *Vp1* alleles on B genome in wheat varieties with distinct pre-harvest sprouting resistance and *ABA* sensitivity. Based on these alleles, a STS marker was developed and validated among a set of Chinese wheat germplasm. Further analysis of EU wheat varieties also revealed a new *Vp1B* allele which doesn't exist in Chinese wheats. Expression of these alleles in *Arabidopsis abi3* mutants clarified their roles in determining the seed dormancy and *ABA* sensitivity. Furthermore, characterization of *Vp1* promoters indicated that each genome has different set of promoter sequence; however, all of them has *ABA* and *GA* response elements and is regulated by *ABA* and *GA* signal. Moreover, the characterization and roles of genes such as *AIP2*, which can interact with *Vp-1*, in seed dormancy and sprouting was also investigated. At last, association studies on the variations of *R* and *DFR* gene with PHS difference of red wheat varieties were also studied. Understanding the gene network involved in PHS would shed light on the mechanism underlying PHS resistance in both white and red grained wheat varieties.

Evaluation of methods of measurement of pre-harvest sprouting resistance in durum wheat

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Pre-harvest sprouting (PHS) occurs when ripe grain germinates in the field in wet weather due to a lack of dormancy. Pre-harvest sprouting is an important problem associated with grain yield loss, reduced end-use quality and lower seed viability for subsequent planting. Thus, PHS resistance is a priority research topic and an important objective in wheat-breeding programs worldwide. The aim of this study was to evaluate the resolution of four methods of measurement of PHS resistance [germination index (GI), germination resistance (GR), sprouting index (SI) and percent germination (PG)] across three time intervals (7, 14 and 21 d) from the start of germination through the ability to identify QTL (Quantitative Trait Loci).

Field experiments over three years and a growth chamber experiment with ninety-eight durum wheat (*Triticum turgidum* L. var *durum*) recombinant inbred lines (RIL) from a cross of a low dormancy line (Sentry) by a moderate dormancy line (Kyle), were conducted to access PHS. Lines were genotyped with 153 markers. The Kruskal–Wallis test ($P < 0.005$) and the interval mapping procedure in MapQTL were performed to identify molecular markers significantly associated with preharvest sprouting resistance measures. The QTL interacted with the environment, with a QTL identified in one environment but not another. The different QTL identified across duration and type of measure indicated that no one measure encompassed all facets of preharvest sprouting resistance. In this sense, to better understand PHS resistance multiple measurements may be required on the same genetic material.

Keywords: dormancy, sprouting index, germination resistance, germination index, percent germination, QTL.

The manipulation of proanthocyanidin biosynthesis in wheat to investigate the role of grain colour in PHS

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Red grain colour in wheat contributes significantly to PHS resistance but the mechanism is poorly understood. The red pigment is proanthocyanidin (PA), a polymerised flavan-3-ol, which we have determined to be comprised exclusively of catechin (rather than epi-catechin) terminal and extension subunits. To investigate the role of PA and its precursors in dormancy, we are developing transgenic lines with reduced expression of key genes from the PA biosynthetic pathway. A mapping population of white grained lines has been developed from a large doubled-haploid population segregating for grain colour, and will be used to identify QTLs for sprouting resistance. Finally, we are characterizing white alleles of each of the homoeologues of the wheat *R* gene present within a wide range of wheat germplasm in order to develop genetic markers that can be used to accelerate the breeding of white-grained varieties suitable for the UK.

Development of molecular markers for sprouting tolerance in spring barley

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Wet field conditions just prior to harvest can cause pre-harvest sprouting in barley (*Hordeum vulgare*) resulting in significant economic losses especially in barley genotypes with low seed dormancy. On-the-other-hand, too much dormancy can cause inconsistent germination, creating problems in the malt house or during crop seeding. Seed dormancy is defined as the failure of viable kernels to germinate under optimum conditions of moisture, oxygen, and temperature. Selection for sprouting resistance in a barley-breeding program is challenging because the dormancy trait is influenced by the environment, and is controlled by multiple genes, that are often linked to important quality traits. Utilizing molecular markers linked to dormancy would be one method of selecting for desirable levels of seed dormancy and sprouting tolerance in barley without the problem of environmental effects on expression, and could be used to break up undesired linkage (repulsion) effects that reduce seed quality. The objective of this study was to identify, map and develop molecular markers linked to genes affecting dormancy and sprouting tolerance in Western Canadian germplasm. A recombinant inbred line (RIL) population was developed by crossing a high dormancy 'Samson' (FCDC 6 row feed barley) derived line with 'TR118' (developed as a malt variety, by Dr. B. Harvey, Saskatoon). Seed dormancy levels were determined using a weighted germination index (WGI) and sprouting tolerance was determined using a spike sprouting index (SSI) on this population of 239 RILs. This phenotyped population was subsequently analyzed with DArT and SSR markers. Several QTLs for seed dormancy and sprouting tolerance were identified across the genome. The strong dormancy phenotype of Samson barley is the result of multiple seed dormancy and sprouting tolerance loci. Major QTLs were located on the 7(5H), 3(3H), 4(4H) and 1(7H). Overlapping QTLs for sprouting tolerance and seed dormancy may represent alternative alleles at the same locus. Molecular markers for QTLs linked to seed dormancy that may benefit a breeding program attempting to improve sprouting tolerance in barley are important.

Allelic differentiation and downstream gene networks of the pleiotropic gene underlying the association between seed dormancy and red pericarp color in rice

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Association between seed dormancy (SD) and red pericarp color (PC) has been reported for wheat, sorghum and rice, and used to select wheat cultivars resistant to pre-harvest sprouting. Some critical questions about the association would include: 1) if it arises from a tight linkage or pleiotropy; and in case of the latter, 2) if alleles functional for the PC have differentiated in an effect on the SD trait; and 3) if the pleiotropic gene regulates the two traits through same or different molecular/physiological pathways. The association was accounted for by the *qSD7-1/qPC7* QTL cluster in weedy red rice, but was not detected in wild and cultivated rice with the red PC character controlled by the *Rc/Rd* genetic system. Map-based cloning delimited *qSD7-1/qPC7* to the *Rc* locus encoding a bHLH family transcription factor and also demonstrated that the locus has additional effects on enhanced ABA accumulation in early developing seeds and seed weight. Sequencing of 14 *Rc* alleles from dormant, red PC genotypes of wild, weedy, and cultivated rice detected ~50 point mutations across the entire gene. Phylogenetic analysis classified these *Rcs* into two distinct groups. Each group includes the *Rc* alleles that have been confirmed for a SD effect by linkage mapping. Thus, it is suggested that all naturally occurring *Rcs* also could behavior as a polygene for SD. Transgenic analysis for an *Rc* engineered from a rice cultivar confirmed a SD effect of the transgene in an *indica* but not in a *japonica* recipient, suggesting that the effect of an *Rc* on germination could be masked by the genetic background. Transcriptomic analysis detected >250 candidate genes differentially expressed between two isogenic lines that differ only at *SD7-1/Rc*. These candidates include 2 and 9 (including *Rd*) structural genes on the ABA and flavonoid biosynthetic pathways, respectively. Based on temporal and/or tissue-specific expressions of the genes, it is postulated that as a polygene, *SD7-1* regulates SD through enhancing an ABA signaling pathway initiated during embryogenesis for a dormancy induction; on the other hand as a major gene, *Rc* controls red PC by coordinately activating the networked genes to synthesize flavonoid compounds in the lower epidermal cells of the pericarp tissue. Due to the pleiotropy, *SD7-1* cannot be used to improve white PC cultivars for resistance to pre-harvest sprouting. *SD7-1/Rc* orthologues are predicted in released corn and sorghum genome sequences but not in available databases for barley and wheat genomes.

Identification of grain dormancy QTL in a barley doubled haploid population derived from non-dormant parents

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Grain dormancy is a mechanism that provides protection against pre-harvest sprouting (PHS) in cereals. Single marker association analysis and composite interval mapping were used to identify quantitative trait loci (QTL) contributing grain dormancy in a doubled haploid (DH) barley population (ND24260 × Flagship) of 321 lines genotyped with 624 polymorphic DArT markers. Harvest-ripe grain collected from three field experiments was germinated over a 7-day period to determine a weighted germination index (GI) for each line. DH lines displaying moderate to high levels of grain dormancy were identified, while both parental lines displayed rapid germination within the first two days of testing. Genetic analysis identified two QTL on chromosome 5H that were consistently expressed across the three environments. One QTL (donated by Flagship) was located close to the centromeric region of chromosome 5H (*qSDFlag*), accounting for up to 15% of the phenotypic variation. A second QTL with a larger effect (from ND24260) was detected on chromosome 5HL (*qSDND*), accounting for up to 35% phenotypic variation. *qSDFlag* and *qSDND* displayed an epistatic interaction and DH lines displaying the highest levels of grain dormancy carried both genes. We demonstrate that *qSDND* in the ND24260 × Flagship DH population is positioned proximal and independent to the well-characterised malt quality region associated with poor dormancy (SD2). Thus, barley breeders could develop cultivars that combine acceptable malting quality and adequate levels of grain dormancy for protection against PHS.

Falling number – serving the grain community for 50 years

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“Millers and bakers are greatly interested in reliable methods for the determination of the amylase activity in flour, in order that the amylase activity might be adjusted to a desired level.” With these words Mr. Harald Perten started his presentation of the Falling Number method at the AACC annual meeting in Minneapolis April 1963. In the end of the 1950s, Mr Harald Perten worked in the Cereal Laboratory of the Swedish Institute for the Crafts and Industries together with Mr Sven Hagberg evaluating means to facilitate the detection of high alpha-amylase activity in grain and flour resulting from sprout damage. The solution they arrived at was the Falling Number method, and ever since the very early 1960s, the Falling Number method has given the grain community crucial information on alpha-amylase activity. Soon after the development of the method and the instrumentation, the method was standardised by ICC, AACC and other standardisation bodies. The method was then internationally spread and accepted in the grain trade and flour industry as a user standard.

Thanks to the technological development added possibilities and supporting tools have been made available to the Falling Number instruments over the years. Also suggested alternative methods have been seen along the line. This presentation will give an overview of the Falling Number method that today - 50 years after its introduction - still is going strong serving the grain community.

Study of the effects of pre-harvest sprouting on the storability, malting quality and brewing performance of three Canadian malting barley varieties

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Variations in germination, malting and brewing performance during storage for Canadian AC Metcalfe, CDC Kendall, and CDC Copeland barley samples were examined. These barley samples were collected from four successive crop years and had suffered different degrees of pre-harvest sprouting damage (PHSD) at harvest. In this study, all the barley samples were stored under three different storage conditions, which simulated the conditions seen in commercial shipping and handling in Canada. During storage all PHSD barley samples showed significant changes in germination energy and water sensitivity; the higher the degree of PHSD the more variation in germination energy and water sensitivity was observed. Barley samples stored at higher temperature showed more variation than those samples stored at a lower temperature. Varietal difference in germination energy and water sensitivity and their interactions with storage conditions were also observed.

In malting trials, at steep the barley samples with higher degrees of PHSD showed faster water uptake and lower chitting rate than the barleys samples with lower PHSD or without PHSD. Barley samples with a high degree of PHSD stored at higher temperatures exhibited lower chitting rates than the samples with the same degree of PHSD stored at lower temperatures. Some varietal differences in water uptake and chitting variations were also recorded. The trial results suggested that germination energy and malting performance were all sensitive to PHSD and storage conditions.

In brewing trials, the malts produced from the barley samples stored up to five months with different degrees of PHSD all performed satisfactorily in the brewhouse. Malts produced with barley that had suffered PHSD and were then stored for several months recorded variations in color, conversion time, brewhouse yield, material yield and attenuation limits. The malts made from barley with a high degree of PHSD tended to record higher wort color, longer conversation time, lower brewhouse yield and lower material yield, as well as lower attenuation limits.

Pre-harvest sprouting and malting quality: which one is more important in barley

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Malting quality is the primary objective of various barley breeding programs around the world as there is a premium for malting quality in many markets. In an effort to further improve malting quality, QTLs/genes for malting barley from around the world have been introduced into Australian barley varieties through marker assisted selection. Introducing the high malting quality QTLs from the Canadian germplasm resulted in release of barley varieties with high susceptibility to pre-harvest sprouting. Multiple populations are developed to map the QTL controlling seed dormancy and pre-harvest sprouting tolerance. QTL were identified on chromosomes 3H, 5HC, 5HL and 7H. Effects of the QTLs varied with environments where the barleys were grown. The QTL of chromosome 5HC is temperature sensitive and has a minor effect in the dry and short growing season environment, but has large effect in the cool and longer growing season environment. Multiple alleles of each QTL were detected using a set of 700 barley accessions from around the world. Negative effects were observed for most of the pre-harvest sprouting tolerance QTLs on malting quality including grain plumpness, protein, extract, malt extract, diastatic power, malting flavour components, activity of alpha-amylase, limit dextrinase, and beta-glucanase. Thus, gene/allele combinations are important to achieve a balance for high malting quality and pre-sprouting tolerance in the different environments.

The QTL on chromosome 3H is controlled by an enzyme involving in the GA synthesis pathway. The deletions in the promoter region and intron 2 resulted in reducing expression of the gene, which in turn reduced the GA content in the developing seeds and increased pre-harvest sprouting tolerance. Efforts are also made to isolate the genes on chromosome 5HC and 5HL. A population of more than 2000 RIL was developed to fine map the genes for pre-harvest sprouting tolerance. BAC clones were sequenced to cover the QTL regions and candidate genes were identified through join analysis of whole genome transcriptome and the BAC sequences. Molecular markers were developed to distinguish different haplotypes of each QTL. A model was proposed to achieve high malting quality and pre-harvest sprouting tolerance for different barley growing regions.

Evaluation of alpha amylase accumulation and falling numbers in soft red and soft white wheat adapted to Michigan

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Michigan has experienced two recent years (2008 and 2009) of severe Pre-Harvest Sprouting (PHS) in wheat. Alpha-amylase is an important component of PHS and the falling number test is used by industry to identify sprouted wheat that is unacceptable for various food products. Red wheat is, in general, more resistant to PHS than white wheat. The objective of this study was to evaluate wheat cultivars adapted to Michigan for the quantity of α -amylase and the corresponding falling numbers at three maturity time-points (before physiological maturity [PM], at PM and post PM) in the absence and presence of PHS inducing conditions. In 2009, twenty soft winter wheat genotypes (10 red and 10 white) with varying levels of susceptibility to PHS were planted in two locations (East Lansing and Clarksville, MI) in a three-replication alpha lattice design. In 2010, twenty-four genotypes were planted in three locations (East Lansing, Saginaw and Ingham). Spikes were collected three days before PM, at PM, and three days post PM. Immediately following collection, samples were frozen and then freeze-dried and threshed. In 2010 a subsample from each plot was artificially misted for 48 hours to induce PHS, while a second subsample was non-misted for the same period of time as a control, after which they were frozen, freeze-dried and threshed. Threshed samples were milled and evaluated for α -amylase activity and Falling Number (FN) values. The 2009 data, in which samples were harvested in non-PHS conditions, showed clear trends in the reduction of α -amylase and the increase in FN during the progression of maturation. In addition, α -amylase and FN data were significantly correlated. Significant differences for α -amylase levels and falling number were found between genotypes within each wheat class (red and white) and at the three mature time points. α -amylase activity levels converged towards similar values at 3 days post PM, though a similar convergence was not observed in FN values. This base level of α -amylase and FN that has been established is revealing in and of itself. The 2010 data, which includes α -amylase and FN in both non-PHS and PHS conditions, will reveal if the base level of α -amylase and FN determined from non-PHS conditions has a predictive value for PHS conditions, and also indicate the overall levels of resistance of PHS in wheat adapted to Michigan. Both 2009 and 2010 data will be presented.

Modifying expression of Thioredoxin to improve preharvest sprouting resistance and other cereal grain properties

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Thioredoxins (Trxs), ubiquitous low molecular weight proteins functional in cellular redox regulation, are particularly important in plants. Work in cereals and a legume has established Trx *h* as a central regulatory protein of seeds through its action in reducing disulfide (S-S) groups of diverse seed proteins, e.g., storage proteins, enzymes and enzyme inhibitors. Extensive evidence indicates that adding Trx, NADPH, and NADP-thioredoxin reductase to flour or seed preparations from a number of cereals causes changes in redox state of storage proteins. Early *in vitro* studies were complemented with engineering approaches. Barley grain engineered to overexpress Trx *h* showed accelerated germination and early or enhanced expression of α -amylase and pullulanase. Other modifications of Trx *h* levels in wheat exhibited (i) enhanced protein solubility and digestibility, (ii) reduced allergenicity of wheat gliadins, and (iii) improved dough quality from poor quality wheat flour. Recently, underexpression of Trx *h9* in wheat endosperm resulted in effects opposite those observed with Trx *h5* overexpression in barley – retarded germination and delayed or reduced expression of associated enzymes. This led to dramatic reductions in preharvest sprouting in the greenhouse and the field – without yield penalties. These observations raise the question of how such changes in the endosperm are communicated within, and possibly outside, the seed. Recent studies on *trx h9*, a membrane-associated Trx*h* capable of moving from cell-to-cell, provide evidence for its role in intercellular communication of redox state.

Association mapping for pre-harvest sprouting tolerance in bread wheat

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In bread wheat (*Triticum aestivum* L.), pre harvest sprouting tolerance (PHST) is a complex trait, for which a number of interval mapping studies led to mapping of QTLs on all 21 chromosomes; however, major QTLs were shown to be present only on chromosomes 3A, 3B, 3D and 4A. Biparental mapping populations, each representing very limited genetic variation due to the two parents, are generally used in all such interval mapping studies. As an alternative and more powerful approach for detection of marker-trait associations (MTAs), association mapping is used, which allows detection of many more QTLs, because a diverse germplasm covering almost entire genetic diversity for the trait is utilized (no mapping population is used). In the present association mapping study, a set of 242 genotypes of wheat including 230 Indian wheat cultivars and 12 exotic accessions were genotyped using 250 simple sequence repeats (SSRs). The population was also phenotyped for PHST for 2-4 yrs using a 1-9 scale, where 1 represents complete tolerance and 9 represents complete susceptibility. Model-based cluster analysis was performed with the help of STRUCTURE 2.3.3 to infer population structure and to define the number of sub-populations. This analysis resolved 15 sub-populations in the above set of 242 wheat genotypes. Marker-trait associations were detected using mixed linear model (MLM) approach with the help of TASSEL, using a P-value of ≤ 0.05 . As many as 15 new markers were found to exhibit significant association with PHST. These markers were present on chromosomes 1B, 2A, 2D, 3D, 4B, 4D, 5B, 6D, 7B and 7D. Out of these 15 markers, 5 markers (cfd2, gwm425, wmc827, gwm325 and gwm251) were present in the four regions already known to carry QTLs for PHST. These regions included the following: (i) Xwmc631-Xcfd223 with QTL QGi.crc-3D, (ii) Xwmc177-Xwmc63 with QTL QPhs.ccsu-2A.2, (iii) Xbarc54-Xgwm55 with QTL QPhs.ccsu-6D.1 and (iv) Xcfd39-Xwmc652 with QTL QPhs.ccsu-4B.1. The remaining ten markers (wmc526, gwm413, gwm558, wmc274, wmc52, wmc48, gwm403, wmc109, gwm455 and barc111) were present in regions which appeared to be different from those reported in earlier studies, since none of them was present in any of the intervals, which carried the QTLs already discovered. These new markers will be validated in future studies and could be used for marker-assisted selection for developing PHS tolerant genotypes. The information generated in the present study may also be used for high-resolution mapping and subsequent cloning of one or more QTL for PHST.

Viviparous-1 gene in the Kazakhstan wheat varieties

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Kazakhstan is the main wheat producer in Central Asia. Spring wheat area takes about 12-13 million ha in the North of Kazakhstan and 1-1.5 mln. ha in the South of the Republic is occupied by winter wheat. Pre-harvest sprouting (PHS) of wheat kernels occurs in Kazakhstan, especially in the Northern regions of the country due to rains during harvesting. PHS results in low yield and reduction in end-product quality. Different levels of PHS tolerance were observed among Kazakhstan wheat varieties. Thus, breeding for PHS tolerant cultivars is important in Kazakhstan.

It is known that the Viviparous (*Vp-1*) gene is a regulator of late embryo development in bread wheat. Based on the alleles, a co-dominant STS marker of *Vp-1B* gene was developed and designated as *Vp1B3*, which in most cases could amplify either 845 or 569-bp fragment from tolerant cultivars, and 652-bp from the susceptible ones. The objective of this study was to evaluate the diversity of the alleles of Viviparous-1B (*Vp-1B*) gene associated with PHS tolerance in the collection of 40 spring and 48 winter wheat varieties from Kazakhstan, 70 spring wheat accessions from 10-th KAZSIB (Kazakhstan and Siberia Network) by using STS marker *Vp1B3* and in order to provide basic information for breeders to produce improved PHS tolerant varieties.

We determined the diversity of *Vp-1B* alleles in North Kazakhstan wheat varieties. Two types of alleles of *Vp-B1* gene: *Vp-B1a*, *Vp-B1c* are met in the studied samples with different frequency. For example, the frequency of *Vp-B1a* in improved Kazakh spring wheat cultivars was 70.6%, *Vp-B1c* – 14.7%; in accession from 10-th KazSib – 59.7% and 26.9% respectively. Cultivars with *Vp-B1(a+c)* made the rest 14.7 and 13.6% of each category. The frequency of alleles *Vp-B1* gene in improved Kazakh winter wheat cultivars sown in the South of Kazakhstan is opposite to studied spring wheat cultivars and accessions sown in the North of Kazakhstan. Allele *Vp-B1c* is met more often than *Vp-B1a*. For example, the frequency of allele *Vp-B1c* in improved Kazakh winter wheat cultivars was 85.1%, *Vp-B1a* – 10.6%.

The results of our investigation indicate the low diversity of alleles of *Vp-B1* gene in improved spring and winter wheat cultivars from Kazakhstan and different their distribution in the Southern and Northern regions of Kazakhstan.

Exploring genetic and epigenetic basis of seed dormancy in cereals

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Epigenetic mechanisms play an important role during fertilization and seed development in plants. Recent studies in *Arabidopsis* have shown that epigenetic modifications of the chromatin are important for ABA mediated developmental processes including seed dormancy. However, there have been very few studies in cereals to explore the role of epigenetic processes in seed development. To investigate the genetic factors involved in chromatin modification during seed development we are characterizing the genes and mutants of RNA-dependant DNA silencing pathway in barley and wheat. Here we present preliminary results for characterization of the Argonaute4 (Ago4) and Dicer Like3 (Dcl3) genes of the DNA silencing pathway. EST and cDNA database search in barley identified two Ago4 like genes, Ago1002 and Ago1003, which are expressed during seed development. Wheat is expected to have six Ago4_9 like genes corresponding to the two barley Ago4 genes. We have obtained three distinct cDNA sequences which correspond to the barley Ago1002 gene. Preliminary studies have shown that these three genes are differentially expressed in the embryo during late seed development. Functional analysis in barley showed that a mutation in one of the components of the DNA silencing pathway results in prolonged seed dormancy. Gene expression data along with mutant characterization suggests that various genes of the RNA-dependant DNA silencing pathway may play an important role in regulation of seed dormancy.

Barley seed dormancy test: in light or dark?

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A lack of seed dormancy in barley (*Hordeum vulgare* L.) causes pre-harvest sprouting, which resulted in downgrade of malting barley to feed. Seed dormancy has been used as an indicator for pre-harvest sprouting susceptibility, as pre-harvest sprouting test is restricted by either environment or equipment. The standard EBC seed dormancy was conducted at 18°C in dark. We also examined the light effect on seed dormancy measurement in this study. Germination test was performed in dark and light on a doubled haploid (DH) population with 180 DH lines derived from a cross between Stirling and Harrington. Seed germination was counted at 24h, 48h and 72h after 4ml water imbibition for 100 seeds. Light reduced germination rate by about 25%. Germination test in dark detected one, two and two QTLs at 24h, 48h and 72h, and they accounted for 58.4%, 62.1% and 29.7% of phenotypic variations, respectively. Germination test in light detected one, two and three QTLs at 24h, 48h and 72 h, which can explain 62.9%, 84.5% and 68.35% of phenotypic variations, separately. QTLs identified from both germination tests were located on barley chromosome 5H, but germination test in light detected one extra QTL at 72 h on chromosome 6H. Furthermore, heritability of seed dormancy was improved by up to 22% in light measurement. Our results consistently demonstrated that germination test in light could 1) detect seed dormancy QTLs more efficiently; 2) explain QTL effects more accurately; 3) detect more QTLs than the dark germination test. Further research is required to confirm if the seed dormancy test in light have better correlation with pre-harvest sprouting tolerance in field.

Panel Session

Daryl Mares (Chair), Ron DePauw, Dave Hatcher, Annelie Barnard

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POSTERS

Analysis of a wheat ABA insensitive 5 (ABI5) homologue, TaABF, which controls seed germination

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In higher plants, the phytohormone abscisic acid (ABA) plays important roles in regulating various processes of plant development, including synthesis of seed storage proteins, promotion of seed desiccation tolerance and dormancy, and inhibition of germination and reproductive growth. Some basic leucine-zipper (bZIP) type transcription factors bind to the ABA response element (ABRE) and regulate the expressions of ABA response genes. In the seeds of *Arabidopsis*, a bZIP transcription factor, ABI5, interacts with VP1/ABI3 and effectively activates the transcription of the ABA induced genes. In common wheat, it has reported some homologues of ABA Insensitive 5 (ABI5) induced by ABA or some stress such as low temperature and drought (Johnson et al., 2002, Kobayashi et al., 2008). One of them, TaABF was isolated a bZIP proteins which was specifically bound to PKABA1 in dormant wheat seeds (Johnson et al., 2002). Based on the comparison of the deduced amino acid sequences of TaABF was classified into group A of a bZIP type transcription factor family in plants and showed highly homology to ABI5 in *Arabidopsis* and OsABI5 in rice. We were interested whether TaABF could control seed germination and whether it would be an ortholog of ABI5 in wheat. In this study, we analyzed the expression patterns of *TaABF* in wheat tissues and the function by using wheat aleurone cells and *Arabidopsis* introduced *TaABF*. *TaABF* mRNA was highly accumulated in embryos of late ripening wheat seeds, especially in the scutella and the coleorhizae, and the expression was induced by ABA. We also performed transient assays in wheat aleurone cells introduced *TaABF* driven by a rice *Actin 1* promoter. The results showed that TaABF could activate the transcription of the *Em* promoter contained the ABRE in the aleurone cells with and without ABA. Moreover, we introduced *TaABF* driven by a CaMV 35S promoter to *Arabidopsis* and analyzed the effects. As the result, the ectopic over-expression of *TaABF* in transgenic *Arabidopsis* was hypersensitive to ABA in seed germination. These results suggest that TaABF enhances the transcription of ABA induced genes contained the ABRE in the promoter and consequently they promote ABA sensitivity in seeds.

Application of PLUG markers to narrow the QTL interval for preharvest sprouting resistance on chromosome 2B in wheat

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PCR-based Landmark Unique Gene (PLUG) markers are EST-PCR markers developed based on the orthologous gene conservation between rice and wheat. Bin-mapped PLUG markers on wheat chromosomes have revealed orthologous relationships among wheat and rice chromosomes, and can be used as anchors for developing markers in targeted regions. In this study, we applied the markers to narrow the region of wheat preharvest sprouting (PHS) quantitative trait loci (QTL) located on chromosome 2B in wheat. The PHS QTL originated in Clark's Cream (CC) and was mapped in a doubled haploid (DH) white winter wheat population from a cross between PHS resistant, Cayuga (derived from CC), and PHS susceptible, Caledonia. The QTL was also mapped to the deletion interval 2BS1-0.53-0.75 in Chinese Spring deletion lines. Recent fine mapping revealed that there are two QTLs closely linked in coupling and one of the QTL intervals was 4.2 cM located between Wmc453c and Barc55. Since a PLUG marker, TNAC1183, was already assigned to the same deletion interval of the QTL, we used the marker as an anchor for developing new markers. TNAC1183 was designed from a wheat EST that showed high similarity to the rice gene, Os07g0628500 (LOC_Os07g43530). Using rice genome sequence information, we designed 120 primer sets and screened for polymorphisms between the two parental varieties. Five markers were polymorphic and all mapped on group 2 chromosomes. Two markers were mapped on each of chromosomes 2A and 2D, while TNAC9025 was tightly linked to Barc55 on 2B. Subsequently, a BC1F5 population which was developed by backcrossing selected doubled haploids to Caledonia was used for fine mapping. To narrow the interval, homozygous recombinant families containing break points in the QTL interval were selected from the population using three flanking markers, Gwm429, Barc55 and Wmc474. From 456 BC1F5 lines, eight recombinants between TNAC9025 and Barc55 were detected. Using these lines, we compared PHS scores that were obtained at two locations; Synder and Ketola, Ithaca, NY during the fall of 2008. Mean scores of six lines with the Cayuga allele for TNAC9025 were 2.40 and 1.53 at Synder and Ketola, respectively. On the other hand, scores of the other two lines with Caledonia were 4.30 and 3.10 at Synder and Ketola, respectively. Since TNAC9025 is designed based on rice gene Os07g0659100 (LOC_Os07g46500), rice gene annotations around the gene provide valuable information for finding a candidate gene related to PHS.

Breeding white-grained variety tolerant to pre-harvest sprouting by marker- assisted selection in wheat

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Because of pre-harvest sprouting (PHS), about 10% wheat was degraded during 2008-2010 in China. Most of wheat cultivars possess little seed dormancy in white-grained wheat planting districts. There are few examples where an understanding of the physiology and the genetics of putative important tolerance- related traits has led to improved tolerance. Seed dormancy evaluated by germination index (GI) is often regarded as a main and pivotal component of observed genetic variation for PHS. Improving seed dormancy can decrease or avoid PHS damage before harvest, but it is a complicated trait controlled by multi-genes and influenced by many environmental factors. Because seed dormancy is difficult to be accurately evaluated under field condition, molecular markers will play an important role in dormancy evaluation. Marker-assisted selection (MAS) has been found useful for improvement of PHS tolerance. Success will first depend on identifying the most important traits in the target regions. It will then depend on accurate and fast phenotyping, which, in turn, will lead to trait-based selection being immediately transferable into breeding operations, and being able to identify the underlying genes or the important genomic regions (QTL). In our study, the SSR markers (Xbarc57, Xbarc294, Xbarc310 and Xbarc321) on the short arm of chromosome 3A were used for genotyping the F4 population of Zhongmai18/Jining13. A white-grained variety was bred and was named Zhongmai911 tolerant to PHS. The GI was 0.12 and 0.18 in 2008 and 2009, respectively.

Changes of embryo sensitivity to ABA during seed after-ripening in wheat

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The level of seed dormancy at harvest maturity is the main component of resistance to pre-harvest sprouting in wheat. Wheat genotypes with enhanced dormancy show increased embryo sensitivity to exogenously applied abscisic acid (ABA), a phytohormone considered to be involved in acquisition of dormancy. The aim of this study was to determine genotype differences in embryo sensitivity to ABA during the period of seed after-ripening. Twenty four winter wheat genotypes together with a highly dormant check RL4137, were grown in the field at two locations in Croatia during 2008/2009 growing season in a randomized complete block design with two replicates. From each plot 40 spikes were harvested at harvest maturity (approximately 14% moisture content on a wet weight basis). Spikes were hand-threshed and the seeds were stored at 20°C to allow after-ripening. Germination tests were conducted at 20°C in darkness with treatments: whole seeds in water (T1), embryos in water (T2) and embryos in 20 μ M ABA (T3). Four germination tests were carried out at intervals of 10 days starting from the harvest maturity until 30 days after harvest maturity. Germinated seeds were counted after three and six days and germination rate, expressed as weighted (by day) germination index (WGI), was calculated for each genotype by treatment combination. Combined analysis of variance across locations revealed a significant effect of genotype (G) and location (L) for WGI for all treatments in all germination tests, whereas G x L interaction was significant for WGI for all treatment by germination test combinations except for T2 and T3 at harvest maturity. Generally, at harvest maturity as well as during the course of after-ripening, dormant genotypes showed higher embryo sensitivity to ABA compared to less dormant genotypes. However, large variation in embryo sensitivity to ABA was found within groups of genotypes with high, moderate as well as low level of seed dormancy in all four germination tests at both locations. Furthermore, differences in embryo sensitivity to ABA between dormant and non dormant genotypes disappeared when the two groups were compared to each other at the same level of embryo or seed dormancy attained through genotype dependent time of after-ripening. In summary, results of the present study indicate that at least part of the differences in wheat embryo sensitivity to ABA during the period of seed after-ripening is the consequence of dormancy status of a seed sample regardless of its genotype (*dormancy per se*).

Characterization of a novel wheat (*Triticum aestivum* L.) mutant with reduced seed dormancy

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A novel wheat mutant (*Triticum aestivum* L.) with reduced seed dormancy, RSD32, was isolated from a NaN_3 -treated population of a dormant cultivar, Norin61. Results show that RSD32 reduced not only the seed dormancy but also sensitivity to abscisic acid (ABA) on germination. The segregation ratio in the F_2 population of a cross that had been derived from Norin61 and RSD32 suggests that the reduced dormant phenotype was inherited as a recessive trait. At 30 days after pollination (DAP30) and later developmental stages, ABA insensitivity of RSD32 was observed, but the seeds were sensitive to ABA at DAP20. Defects of ABA sensitivity depended upon seed development. Auxin acted synergistically for sensitivity to ABA in germination of Norin61, but the effects of auxin on ABA sensitivity were diminished in RSD32. The expression of *TaABF1*, a bZIP class transcription factor associated with ABA signal transduction in seed, was lower in embryos of RSD32 than those of Norin61. Relative amount of transcripts of *TaABF1* was also determined in wheat cultivars possessing different levels of seed dormancy and ABA sensitivity. Zenkoujikomugi and OW104 were dormant cultivars showing high ABA sensitivity similar to Norin61. Chihokukomugi, RL4137 and Chinese Spring, all non-dormant cultivars, show low sensitivity to ABA. Tamaizumi, AUS1408 and Kitakei-1354 represent intermediate levels of seed dormancy and sensitivity to ABA. The relation between seed dormancy and ABA sensitivity is high ($r = 0.89$) in these cultivars. The expression of *TaABF1* revealed high coefficients of correlation to seed dormancy ($r = 0.78$) and ABA sensitivity ($r = 0.86$). *TaDOG1* is a wheat orthologue of *Arabidopsis DOG1*, which is identified as a quantitative trait locus (QTL) for the regulation of seed dormancy. Moreover, RSD32 showed lower expression of *TaDOG1* in embryos. Both *TaABF1* and *TaDOG1* have potential functions for regulation of wheat seed dormancy.

Development of winter and spring wheat with excellent resistance to pre-harvest sprouting

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Pre-harvest sprouting and high alpha-amylase activity are the most serious problems for the wheat production and quality in Hokkaido, the northern most part of Japan. In 2008, rain continued at harvest time in most districts, wheat production suffered large rain damage the average yield was reduced to 74% of normal production. It has become more frequent for wheat to be exposed to continuous rain for several days in harvest time. In addition, cool condition, i.e., below 15 °C, combined with rainfall, accelerates the damage of pre-harvest sprouting. The most effective means of controlling this damage is by breeding resistant cultivars. Selection and evaluation of pre-harvest sprouting of resistant winter and spring wheat lines were conducted by following tests: continuous rain treatments of intact spikes at 15°C and germination tests of hand-threshed grains at 10°C. The spikes and grains were sampled at maturing time and several days after maturing. A soft red winter wheat line, Kitakei(KK) 1838, with excellent resistance to pre-harvest sprouting, has selected from doubled haploid lines raised by anther culture from the cross W148-59-8(OW104)/98046//99015. OW104 is highly sprouting resistant and 99015 is good quality for Japanese noodle. KK 1838 hardly sprouted and maintained high falling number (> 300 s) through 7 days continuous rainfall treatment at low temperature. The germination rate of late harvested sample of KK 1838 was low. KK 1838 has tolerant to snow mold, good flour color, and good quality for Japanese noodle. A hard red spring wheat line, Kitakeiharu(KKS) 823, with high dormancy, was bred from the cross 17S6 and KKS775. 17S6 is sprouting resistant and KKS775 is scab resistant. KKS 823 hardly sprouted through 8 days continuous rain treatment at 15 °C. The germination rate of late harvested sample of KKS 823 was very low at 10 °C. KKS 823 has tolerant to logging, resistant to Fusarium head blight, and good quality for bread.

GA sensitivity of developing wheat grain in relation to late maturity α -amylase

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Late maturity α -amylase (LMA) in wheat is a genetic defect which results in synthesis of high pI α -amylase during the middle stages of grain development in the absence of pre-harvest sprouting. Such grains retain unacceptable levels of α -amylase activity at maturity and may not meet the falling number receival standards. The incidence of LMA is strongly influenced by environment and the presence of semi-dwarf, GA insensitivity genes. Semi-dwarf (*Rht1* or *Rht2*) LMA genotypes require a cool temperature shock during the middle of grain development to initiate LMA expression and the level of expression is reduced relative to tall LMA genotypes. These observations, together with the established role of GA in synthesis of α -amylase by aleurone tissue, suggest underlying mechanisms of LMA involve a change in GA and/or GA sensitivity. This study compared GA sensitivity of developing grains of tall *rht* and semi-dwarf *Rht1* and *Rht2*, LMA and non-LMA genotypes. GA sensitivity developed earlier in tall LMA genotypes compared to tall non-LMA genotypes and at the time which corresponded to LMA expression. In semi-dwarf genotypes a similar increase in GA sensitivity only occurred in LMA genotypes that were subjected to a two day cool temperature shock. The results here provide important knowledge on the role of GA sensitivity in the mechanisms underlying LMA.

Identification of QTL for seed dormancy in the spring barley CAP lines

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Seed dormancy is a mode of developmental arrest, which prohibits viable seed from germinating under favorable conditions. Dormancy is considered an adaptive trait promoting the survival of the organism. Dormant genotypes need a prolonged storage time before malting, which increases the probability of seed decay if problems during storage occur. In contrast, low dormant genotypes are more prone to pre-harvest sprouting, which affects seed viability and can make the grains unsuitable for malting. Dormancy tests were performed on 3,072 spring growth habit barley lines from eight breeding programs participating in the USDA-NIFA Barley Coordinated Agricultural Project (CAP). Dormancy was determined on seeds harvested at physiological maturity (Zadocks 8.9) and stored at -20°C until tests were done. Genotype data on 3,072 SNPs were collected on the lines using the Illumina GoldenGate Assay. Both phenotype and genotype data were subjected to association mapping (AM) analyses using the computer software TASSEL and a mixed linear model with population structure estimated by principal component analysis and kinship. Separate AM analyses were done for each year. Within a year, AM analyses were done separately for each breeding program and across breeding programs. QTL regions were found on all seven chromosomes, but the most common were ones in the telomeric region on the long arm of chromosome 5H (5HL), the centromeric region on chromosome 5H, and the short arm of chromosome 4H. The QTL region in chromosome 5HL was consistently observed across seven of the eight breeding programs in 2006, and less obvious for the years 2007 and 2008. Other QTL were specific to each breeding program and year, which is evidence of the high influence of environmental factors over the expression of genes involved in dormancy. Common SNPs that could be used for MAS across breeding programs were found only on chromosome 5HL. Because of the relationship between alpha-amylase activity and pre-harvest sprouting, additional, analyses on marker-trait association between dormancy and alpha-amylase levels are currently ongoing and will be discussed.

Identification of recombinant inbred barley lines combining pre-harvest sprouting resistance of the Australian cultivar 'Baudin' with Canadian malting quality

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Pre-harvest sprouting (PHS) is a problem for many malting barley cultivars when wet conditions occur prior to harvest, but too much seed dormancy (or PHS resistance) can be detrimental in the malt house. Australian cultivars 'Chebec' and 'Stirling' possess a major quantitative trait locus (QTL) that controls PHS resistance on chromosome 5HL, while the Canadian cultivar 'Harrington' has an allele at that locus that not only favours susceptibility to PHS but also increases malt extract, diastatic power, alpha amylase and free-amino nitrogen, making it difficult to develop high malting quality cultivars with PHS resistance. This study was undertaken to determine if any recombinant inbred lines (RIL's) from the 'Baudin'/TR253 cross combined Baudin's PHS resistance with Canadian two-row malting barley quality from the advanced breeding line TR253. An experiment was conducted over 6 site years with 26 RIL's carrying the Baudin allele for PHS resistance on chromosome 5HL, 26 RIL's carrying the TR253 allele for susceptibility and high malting quality, 2 parents, and 6 other cultivars differing in PHS resistance and malting quality. Although most of the RIL's with the Baudin allele had poorer malting quality overall, there were several that approached TR253 and 'AC Metcalfe' in malting quality.

Influences of grain bracts and embryo on resistance to pre-harvest sprouting on bread wheat genotypes

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Pre-harvest sprouting (PHS) is one of the major constraints of wheat production in across the world and reduces grain yield and quality. This experiment was done in order to assessment of PHS resistance of 7 wheat promised lines. After physiological maturity, the spikes of these lines were transferred to laboratory. Extracts of grain bracts and embryo of these lines were prepared to assessment of effects of materials inside of them on PHS. For this mean, the grain of test cultivar which is susceptible to PHS, examined by this extracts. During different germination test, traits include of rootlet number, rootlet length, shoot length, rootlet wet weight, shoot wet weight, rootlet dry weight, shoot dry weight, rootlet/shoot dry weight, shoot/rootlet length and α -amylase rate were noted. The data of this experiment were analyzed by SAS and Germin softwares. Results of this study showed that these lines have a significant difference in resistance to PHS.

Keywords : pre-harvest sprouting, wheat, bracts, embryo

Late maturity α -amylase: high pI alpha-amylase synthesis during grain development in wheat

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Late maturity alpha-amylase (LMA) is a genetic defect present in particular wheat genotypes that can result in the untimely synthesis of high pI alpha-amylase during grain development. Whilst LMA shows some similarity with germination or GA-stimulated alpha-amylase synthesis in aleurone, the mechanisms and genes involved in LMA have not been identified. A major QTL on the distal end of chromosome 7B and a minor QTL in the centromeric region of 3B were shown to be associated with LMA in Cranbrook (LMA) x Halberd (non-LMA). Targeted genotyping in populations involving several other sources of the defect confirmed that the 7B QTL plays a major role in genetic control of LMA in many LMA-prone genotypes. The 7B QTL was reported to be mapped very close to the *Bo1* locus by Schnurbusch et al. 2007 in a Cranbrook x Halberd doubled haploid population. LMA phenotypic data for lines recombinant in the *Bo1* region located the 7B LMA QTL to the same 1.8cM genetic interval as *Bo1*.

Marker assisted development and evaluation of near isogenic lines for seed dormancy genes on chromosome 3A and 4A in bread wheat

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Seed dormancy is one of the most important factors involved in pre-harvest sprouting tolerance in wheat, because lack of seed dormancy often causes serious degradation of cereal quality when seeds are matured in wet weather conditions. A lot of quantitative trait loci (QTL) controlling seed dormancy have been reported so far, two of them on chromosome 3A and 4A are known to be major QTL with relatively large effect. In order to analyze the gene action of the two loci and introgress the loci into elite varieties, near isogenic lines (NILs) were developed by marker assisted selection (MAS) derived from a cross between the highly dormant line OS21-5 (OS) and Japanese elite variety Haruyokoi (HK) as the recurrent parent with an insufficient level of seed dormancy. Four genotypes of NILs, both loci with OS homozygous alleles (3A4A NILs), either locus on 3A or 4A with OS homozygous allele (3A NILs, 4A NILs), and both loci with HK homozygous alleles (null NILs), were selected by MAS and evaluated for seed dormancy under greenhouse conditions. The germination behavior of the homozygous NILs showed that both loci on 3A and 4A contribute to maintain seed dormancy after maturity but do not affect immature seed germination before maturity. Furthermore, the seed dormancy of 3A4A NILs was clearly strong compared with those of 3A NILs and 4A NILs, therefore the two loci were shown to have genetically additive effect each other.

Multiple alleles of chromosome 5HL associated with seed dormancy and pre-harvest sprouting in barley

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Quantitative Trait Loci (QTL) analyses demonstrated that the terminal region of barley chromosome 5HL is the most important gene controlling seed dormancy and pre-harvest sprouting in the Australian environment. Six isogenic lines were developed to contain the chromosome 5HL alleles from the Australian, European and North American barley germplasm. Genotyping the 6 isogenic lines using more than 600 DArT markers demonstrated that these lines were only difference on chromosome 5HL. Seed dormancy and dormancy release were measured in consecutive 11 weeks after harvest. The isogenic lines showed at least 5 different seed dormancy profiles. Comparative analysis established that the terminal region of barley chromosome 5HL is syntenic to rice chromosome 3L near the telomere end and chromosome 1 of Brachypodium. The rice BAC (Bacterial Artificial Chromosome) sequences covering the region of chromosome 3L and gene sequences of Brachypodium were used to search barley expressed sequenced tags database. Thirty-three genes were amplified by PCR (polymerase chain reaction) with the primers designed from barley ESTs (expressed sequence tag). Comparison of the sequences of the PCR generated DNA fragments revealed polymorphisms including single nucleotide polymorphism (SNP), insertions or deletions between the barley varieties. Ten new PCR based molecular markers were developed and mapped within 10 cM in multiple mapping populations. The mapped genes maintain the micro-syntenic relationship between barley, rice and Brachypodium. The combination of the molecular markers could identify unique haplotypes among the isogenic lines. These gene specific markers provide simple and efficient tools for germplasm characterization and marker-assisted selection for barley malting quality, and ultimately lead to isolation and identification of the major gene(s) controlling multiple quality traits on barley chromosome 5HL.

QTL × environment interaction for malt α-amylase activity in a barley doubled-haploid population

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Pre-harvest sprouting (PHS) can be a problem, particularly in malting barley (*Hordeum vulgare* L.) production. The main consequences of PHS include (i) lower yield due to harvest losses and (ii) reduced quality of end-products. Some studies show that the same quantitative trait loci (QTL) are identified for both PHS α-amylase activity (AA) and malt AA. This information is important because it is often difficult to measure PHS AA due to low level of enzyme activity and high level of starch in extracts of the flour. This presentation reports co-occurrence and stability of QTL controlling malt AA in a barley doubled-haploid (DH) population that was evaluated in nine environments across North America. This population consisted of 150 DH lines developed from a cross between two malting barley varieties (Steptoe × Morex) for the North American Barley Genome Mapping Project (NABGMP). Malt AA was measured on a composite 400-g sample of each DH line and parent. Linkage analysis enabled a total of 223 RFLP (restriction fragment length polymorphism) makers to be mapped onto the seven barley chromosomes covering a total genetic distance of 1221.9cM. We used composite interval mapping (CIM) and Bayesian statistical approach to detecting single QTLs and epistatic QTLs for individual environments. A common QTL was counted if it co-occurred in a pair of environments. Thus, the level of QTL × environment interaction was assessed based on the total frequency of co-occurred QTLs for all possible pairs of 9 environments $[(9 \times 8) / 2 = 36]$. Malt AAs varied from 24.1 in Klamath Falls, Oregon (1991) to 32.1 in Bozeman, Montana (irrigated condition 1992). We identified 49 QTLs across the 9 environments: 40 in one environment only and 9 in two or more environments, with 16, 10, 3, 6, 6, 1 and 7 QTLs detected on chromosomes 1, 2, 3, 4, 5, 6 and 7, respectively. These results indicated strong QTL × environment interaction for malt AA. Marker-assisted selection for controlling malt AA and thus for improving the resistance to PHS should be carried out for specific, target environments.

Relationships between genotype, grain color, and α -Amylase quantification in a red x white mapping population of wheat

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Pre-harvest sprouting (PHS) is the precocious germination of grains in the ear following physiological maturity. It usually occurs during prolonged cool wet condition, and was severe in Michigan in 2008 and 2009. PHS reduces wheat flour quality, which is a critical concern of millers and end users and also results in discounts for farmers. Red wheat is categorically more resistant to PHS than white wheat, but the mechanism behind this relationship is still unclear. Red seed coat color is controlled by three homologous genes on chromosome group 3. In this current study, our goal is to determine the contribution of each of the three homologous genes to grain color and PHS resistance. To do this, a red ($R_1R_1R_2R_2R_3R_3$) x white ($r_1r_1r_2r_2r_3r_3$) spring wheat Recombinant Inbred Line population is being evaluated for grain color, α -amylase level and falling number values with and without PHS inducing conditions. In 2010, the population was grown in a 3 replication alpha-lattice design in Mason, MI. The population contains all eight combinations of gene (R_1 , R_2 , or R_3) x allele (R or r) with 18 to 23 lines within each group. Grain samples were harvested at 3 days after physiological maturity and misted for 48h, immediately frozen (-20 C) and then freeze-dried. Grain color was assessed, and samples were ground for α -Amylase quantification following AACC method 22-02. Preliminary data from this first year (of three) of phenotyping shows significant differences in both grain color and α -amylase levels for the different gene x allele groups.

Role of embryo extract on resistance to pre-harvest sprouting on bread wheat genotypes

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Detection and introduction of resistant cultivars to pre-harvest sprouting (PHS) is one of suitable strategies to reduce clutches in bread wheat production. In this mean, introduction of suitable screening methods with high precision can help to researchers in assessment of genotypes in gene banks. In this experiment, seven promised bread wheat lines after physiological ripening were transferred to laboratory to assessment of resistance of them to PHS. Then, embryo extracts of these lines were prepared and were tested on the test cultivar which be susceptible to PHS. Results of this experiment showed that genotypes numbers 4 and 6 are most resistant and genotypes numbers 1 and 5 are most susceptible to PHS. Also, this experiment showed that materials inside wheat grain embryo, have important roles in creation of resistance or susceptibility to PHS.

Keywords : *pre-harvest sprouting, wheat, embryo.*

Study of effective traits on pre-harvest sprouting of bread wheat genotypes

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In the world, more than 27 million ha of cereals has pre-harvest sprouting (phs) problem. Harvesting season rainfall in these farms caused 10 -50% lost in grain yield. This experiment was carried out in order to evaluation of phs in 7 promised bread wheat genotypes at Gorgan agricultural research station of Iran in 2009-2010 cropping season. For this mean, to assessment of resistance or susceptibility of these lines to phs, after physiological ripening, rates of germination inhibitors and inducers in extracts of embryo and bracts measured in embryo and bracts of these lines in laboratory. Results showed that lines numbers 1 and 7 were the most susceptible and resistance to phs respectively. In addition to results of this experiment showed that materials in bracts more effective than those embryo.

Keywords : pre-harvest sprouting, wheat, bract, extract

The effect of germination temperature on seed dormancy in Croatian-grown winter wheats

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The level of seed dormancy at harvest time is the main component of pre-harvest sprouting resistance in wheat and it is controlled by genetic as well as environmental factors. Temperature is an important environmental factor, influencing the induction of seed dormancy during seed development and maturation as well as the expression of seed dormancy during germination. The aim of this study was to determine genotype differences in expression of seed dormancy at different germination temperatures. Twenty five winter wheat genotypes including a highly dormant check RL4137, were grown in the field at two locations in Croatia during 2008/2009 growing season in a randomized complete block design with two replicates. From each plot 30 spikes were harvested at harvest maturity (approximately 14% moisture content on a wet weight basis). Three germination tests were conducted with hand threshed seeds under controlled environment at temperatures of 15, 20 and 25°C in darkness. The first germination test was started immediately after harvest (Time 1), whereas the second and third germination tests were conducted after 10 (Time 2) and 20 (Time 3) days of seed after-ripening at 20°C, respectively. Germination rate, expressed as weighted (by day) germination index (WGI), was calculated for each genotype by temperature combination. Combined ANOVA across locations revealed significant differences among genotypes (G) and locations (L) as well as significant G x L interaction for WGI for all three germination tests (Time 1, Time 2 and Time 3) and germination temperatures (15, 20 and 25°C). Genotypes responded with decreasing WGI mean values (increasing dormancy), as temperatures changed from 15 to 25°C in all three germination tests. The number of genotypes with the same level of seed dormancy as dormant standard RL4137 decreased from Time 1 to Time 3 at all three germination temperatures. In all three germination tests the number of genotypes ranking similar to standard RL4137 was lower at 25°C compared to other two germination temperatures. At 15°C the ranges of genotypic WGI mean values decreased from Time 1 to Time 3, at 20°C ranges of WGIs were similar for 15 and 20°C and lower at Time 3, whereas at 25°C ranges of WGIs increased from Time 1 to Time 3. This indicates that maximum differences in dormancy between genotypes included in the current study occurred at different stages of after-ripening depending on germination temperature.

The effect of seed priming on improvement of germination in six wheat cultivars under drought stress

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One of the most effects of drought on plant growth is the reduction in seed germination. The effect of Seed priming is the improvement of seed germination. In order to study the effect of seed priming on the improvement of seed germination, an experiment was conducted in factorial form using a completely randomized design with four replications. In this experiment six cultivars of wheat (*Kohdasht*, *Zagros*, *Sabalan*, *Akbari*, *Cros sabalan*, *Rooshan*), five seed priming as: Distilled Water, KH_2PO_4 , PEG 6000, NaCl 2%, CaCl_2 2%, Water were included. The seeds of wheat were placed in priming solution for 12, 24, 36, 48, hours. The result shows that, there were significant difference among the variety of wheat, priming solution, and the interaction between them. The best priming for percentage of germination was KH_2PO_4 , for the root length was NaCl, and for the dry weight of root was CaCl_2 . There is no significant difference between priming and no priming for shoot length. The highest degree of moisture absorb belonged to KH_2PO_4 in 12 hours. More than 12 hours was not useful for improving indices germination. For *khohdasht* and *Sabalan* varieties, the best priming for root length was KH_2PO_4 , and for *Akbary* variety was Nacl, and for *Zagros* was water. Nacl was the best priming for improving shoot in all varieties except *Roshan*. In *Roshan* Water was the best priming.

Key words: wheat, germination, priming

ABSTRACTS NOT PRESENTED

Features of the regulation of amylase maturing grains wheat by gibberellic acid

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Amylases are a key enzymes of starch conversion in cereal seeds. These enzymes largely determine biological and technological quality of grain. The aim of our work was to study the effect of the hormone GA₃ on the induction of amylase synthesis in wheat grains of different phases of maturation.

We used seven varieties of spring wheat (*Triticum aestivum L.*), cultivated in Kazakhstan (Saratovskaya 29, Kazakhstanskaya 10, Almaken, Kaiyr, Scarlet, Lutescens 70, Lutescens 157). Collect seed and analysis of amylase activity was carried out at 25, 30, 35, 40 and 45 days post anthesis (DPA). In the experimental variants grains were incubated for 72 hours in the presence of 10 mM GA. Served as a control seeds, incubated in medium without hormone. Seeds of control options after the 25 DPA to all varieties had similar IEF spectrum of amylases having from 10 to 12 components (mostly β- amylase). Incubating grains this stage of development in the presence of GA led to weak activation of the enzyme and the appearance in the spectrum only 2 additional zones of activity. However, in grains 35 and 40 DPA observed very strong variations in the level of amylase activity, and their composition. Treatment of GA induced about 7-8 new features characteristic of germinating grains. These components were identified as typical of α-amylase germination. Based on these data, we can conclude that significant differences in wheat grains of different developmental stages in the perception of GA signal. Seeds of later phases of maturation (after 35 DPA) are much more sensitive to the hormone and are able to induced α-amylase. This study identified significant varietal differences in the level of activation of isoenzymes α-amylase. This opens up new possibilities in testing varieties for resistance to pre-harvest sprouting.

Genetic variation of *Vp1* in Sichuan wheat accessions and its association with pre-harvest sprouting response and seed dormancy

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Both spring and winter wheat regions in China are affected by Pre-harvest sprouting (PHS). Sichuan lies in southwest China, where the most of rainfall occurs during April to September when wheat is harvested. The present investigation was conducted to identify the allelic variability of *Vp1*, a gene that plays a role in maintenance and induction of dormancy, among Sichuan landraces and recent cultivars with different dormancy levels and to find potential sources of PHS resistance for breeding. Sichuan landrace and cultivar wheat accessions had a wide range of dormancy levels. The average germination index (GI) of Sichuan landrace accessions was 0.232, whereas it was much higher for cultivars at 0.674. The different dormancy levels between landraces and cultivars indicated that pre-harvest sprouting resistance might have been neglected in recent Sichuan wheat breeding programs. The average GI of white grained accessions was higher than for red grained accessions. Specific *Vp-1B* gene alleles were characterized between landraces and cultivars accessions and between white and red grained accessions. The results indicated that *Vp-1B* markers could be used to distinguish cultivars and landraces. Significant relationships between certain *Vp-1B* alleles and GI of Sichuan wheat accessions were shown by Spearman's rank correlation analysis.

Abstracts not presented

Molecular evolution of α -amylase and its inhibitor genes with association of seed dormancy in wild barley

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α -Amylases hydrolyze internal α -1,4-glucosidic bonds in starch and related dextrans and oligosaccharides. Two types of α -amylase inhibitor were detected in cereals. D-type (defense) and R-type (regulation) inhibitors inhibit exogenous insect/mammalian α -amylase and endogenous cereal α -amylase, respectively. The R-type inhibitors are directed against the activity of α -amylase that is synthesized during seed germination. Endogenous α -amylases play an essential role in the germination and malting process by hydrolyzing starch granules present in the endosperm. Wild barley (*Hordeum spontaneum*) was used to study the co-evolution events of α -amylase (*amy1* and *amy2*) and R-type α -amylase inhibitors (*Isa*) under different environments. A total of 16, 48, and 36 SNPs were detected in the mature protein-coding sequence of *Isa*, *amy1*, and *amy2*, respectively. Some population-specific SNPs were also predicted to make amino acid changes in the functionally important protein domains that can determine enzyme-inhibitor and enzyme-starch granules binding efficiency. Highly significant correlations were found between diversity at the gene (*Isa*, *amy1*, and *amy2*) locus and key water variables. The pattern suggests that selective sweeps in the wetter climates, and weaker selection or diversifying selection in the dryer climates results in higher gene diversity. Adaptive evolution of *Isa*, *amy1*, and *amy2* were detected in Israeli wild barley. Moreover, the germination index was much higher in wild barley collected from two mesic climate sites than that in populations from the xeric climate. Correlation among enzyme, enzyme inhibitor, dormancy level, and environment factors are under characterization now.

Role of effects of dormancy bracts and embryo seeds of bread wheat genotypes on pre-harvest sprouting

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Wheat is the world's third largest food crop, and is relied upon as a food source by millions of people. Securing the supply of wheat is a problem because it is susceptible to many biotic and abiotic factors that limit production. One such factor, sprouting of the grain in the head, because of untimely rainfall prior to harvest, is a substantial problem worldwide. Pre-harvest sprouting has a significant impact on wheat growers, who suffer considerable economic hardship as a result of yield loss during harvesting and subsequent downgrading of their sprouted crops. Wheat processors are also affected by this problem, because sprouted grain has significantly altered chemical properties, making it unsuitable for its intended purpose, and often rendering it suitable for animal consumption only. This study investigated mechanisms of dormancy, in wheat genotypes, to assess their suitability for use in hexaploid (bread) wheat to prevent pre-harvest sprouting. A soluble germination inhibitor was found in the bracts (palea, lemma and glumes) surrounding the grain of wheat. Fractionation of an aqueous extract from the bracts, Extraction of lemma and palea of wheat showed that in order var3 and 6 were most tolerant and susceptible to preharvest sprouting. Also extraction of seed embryo showed that var 4 and 6 were tolerant and var 1 and 5 were susceptible to preharvest sprouting and it will be related to high content of germination inhibitory or low enzymes that important in broken seed dormancy.

Key words: dormancy , preharvest sprouting, wheat

The aldehydeoxidase activity and the content of molybdenum is the index of resistant to pre-harvest sprouting in the grains of diverse wheat genotypes

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The tolerance to pre-harvest sprouting of seed is determined genetic features of wheat. The dormancy phenotype in turn is determined by a complex interaction between the genotype and a number of environmental factors that include water supply to the plant and air temperature. The many investigations show that grain dormancy the major component of resistance to pre-harvest sprouting which is provided of endogenous ABA content. The aldehyde oxidase (AO) involved in the biosynthesis ABA. In the catalytic center of this enzyme is included a molybdenum cofactor (Mo-co). Established that the mutants of tomato and barley deficient Mo-co, almost unable to synthesize ABA. It was investigated 12 diverse wheat genotypes, cultivated in Kazakhstan. It was established AO activity and was shown the component composition of AO spectrum by native electrophoresis and the content of molybdenum. The treatment of plants with various concentrations of Mo resulted in increased levels of endogenous ABA and Mo of wheat varieties. Germination and endogenous ABA content of wheat seed directly showed that increasing the concentration of Mo leads to a decrease in seed germination and α -amylase activity. Also it was studied the influence of humidity and lower temperature, on the tolerance grain to germination of the 12 cultivars of wheat, cultivated in Kazakhstan. The wheat seeds of 12 cultivars were placing in controlled-climate chamber with 80% humidity, day 27°C and night 10° C. The native electrophoresis carried out for determine AO activity. It was shown that to 3th day of experiment the AO activity has decreased but to 7th day is decreased more in 3 -6 times in seeds of susceptible wheat to sprouting . The high AO activity kept the four cultivars of resistant wheat to 10th day. The obtained data indicate the significant genotypic variability in AO activity at presented of 12 cultivars of wheat. It is shown that in the conditions with low temperature at night and high humidity at day is revealed genotypes of resistant and susceptible wheat to sprouting.

Key words: Wheat, Aldehyde oxidase (AO), molybdenum (Mo), Preharvest sprouting (PHS), ABA, α -amylase,