

Application of genomic technologies for wheat biodiversity conservation

Primjena genomske tehnologije u očuvanju biodivergentnosti pšenice

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Wheat

most widely cultivated crop on Earth



- ✓ grown on 6 continents
- ✓ 761.5 million tons produced in 2018 on 220 million hectares (mainly EU, China, India, Russia and US)
- ✓ majority of the wheat produced is consumed by humans – 67%
- ✓ 20% of all proteins, 19% of all calories
- ✓ essential calorie intake crop for nearly 2.5 billion people who live on less than 2€ per day
- ✓ No.1 food consumed in the world – average 65kg per person per year
- ✓ projected world population by 2050 is 9.6 billion (UN, 2015)



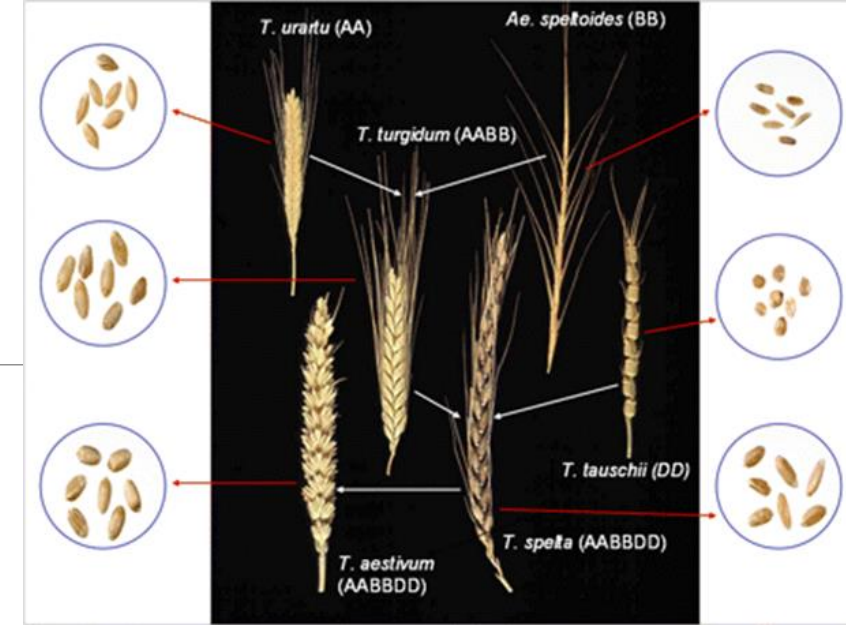
crucial crop for food security



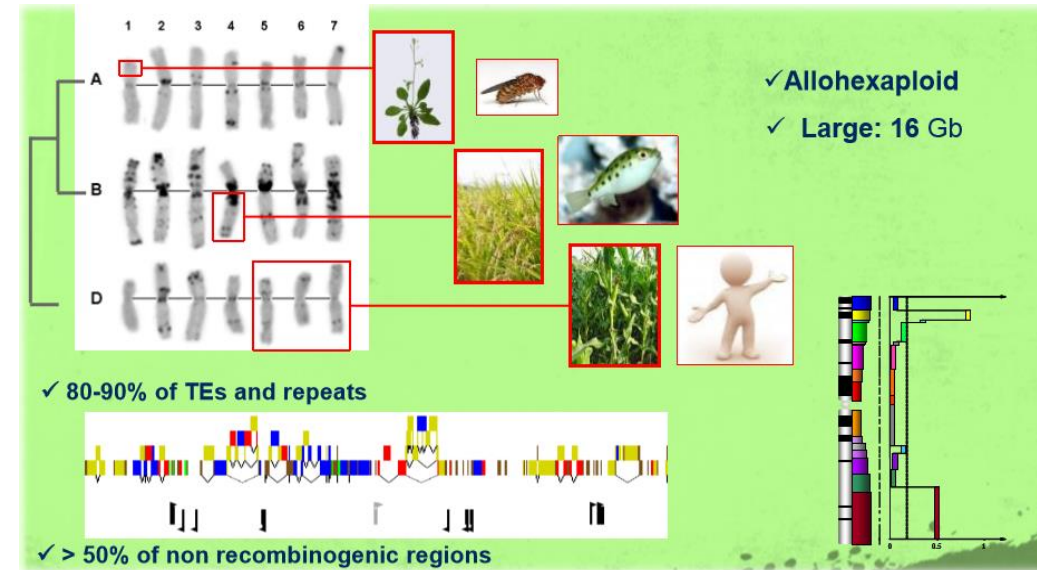
Wheat

biodiversity in a polyploid context

- ✓ Fertile Crescent, 10 000 years old
- ✓ mix of three separate subgenomes
- ✓ $2n (6x) = 42$, allohexaploid, genomes A, B and D
- ✓ *Triticum aestivum* L. ssp. *vulgare* – bread wheat
- ✓ winter (vernalisation) and spring genotypes
- ✓ over 25 000 types
- ✓ many genes are present as two or three functional homoeologous copies
- ✓ **16 Gb, >85% repetitive sequence**



(Shewry, 2009)

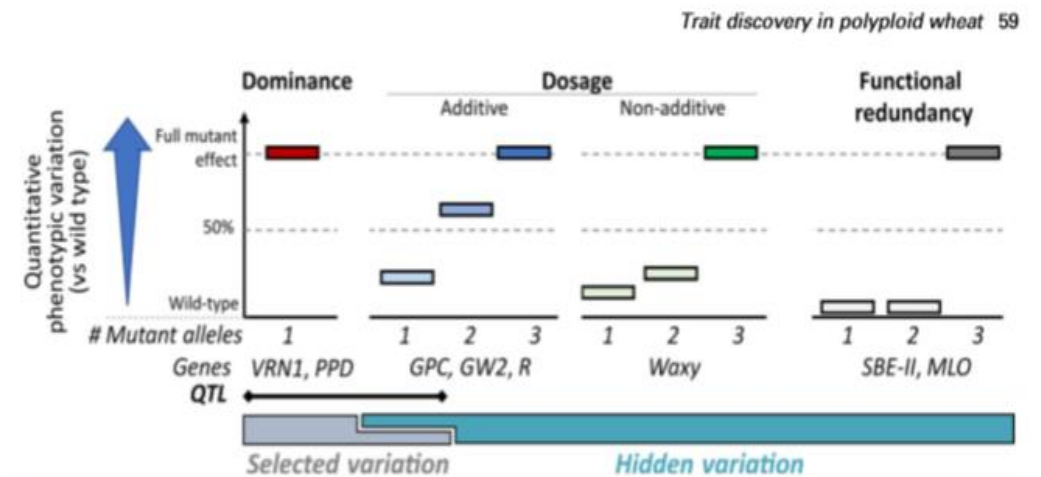


(Feuillet, 2016)

Wheat

biodiversity in a polyploid context

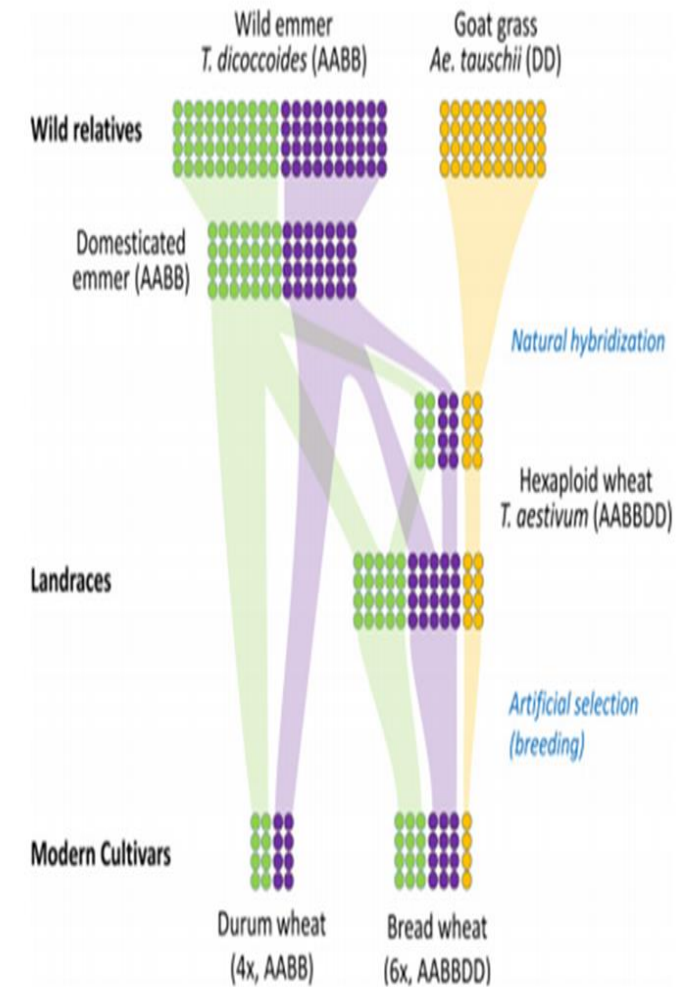
- ✓ A, B and D genomes – set of complementary genes (homoeologs)
- ✓ phenotypic consequences of variation at single locus
- ✓ masked by redundant copies on other homoeologous genomes
- ✓ dominance – vernalisation genes *VRN1* (Yan et al. 2003), photoperiod genes *PPD* (Wilhelm et al, 2009)
- ✓ additive (GPC – grain protein content (Avni et al., 2014); *GW2* – grain weight size (Wang et al., 2018b); *R* – red pericarp colour (Himi et al., 2011))
- ✓ non-additive (*Waxy* genes, Kim et al., 2003)
- ✓ functional redundancy – amylose content (*SBE-II*); recessive disease resistance (*MLO*)



(Borill et al., 2019)

Wheat biodiversity in a polyploid context

- ✓ wild relatives vs. pasta and bread wheat <30% reduced genetic variation
- ✓ A and B genomes – around 30% of the genetic diversity present in the wild emmer progenitor
- ✓ D genome - <10% of the genetic diversity found in goat grass (Haudry et al., 2007; Halloran et al., 2008)
- ✓ causes: domestication bottleneck, selection pressure during breeding (Gaut et al., 2018) and reproductive isolation between closely related species of different ploidy levels
- ✓ reduced diversity in modern wheat - urgent needs for the use of induced and natural variation to provide novel genetic variation, which has been so far unexplored in modern breeding programmes (Winfield et al., 2017)



(Dvorak et al., 2006; Sutherland and Galloway, 2016)

International Wheat Genome Sequencing Consortium - IWGSC 8/16/2018

RESEARCH

RESEARCH ARTICLE SUMMARY

WHEAT GENOME

Shifting the limits in wheat research and breeding using a fully annotated reference genome

International Wheat Genome Sequencing Consortium (IWGSC)*

INTRODUCTION: Wheat (*Triticum aestivum* L.) is the most widely cultivated crop on Earth, contributing about a fifth of the total calories consumed by humans. Consequently, wheat yields and production affect the global economy, and failed harvests can lead to social unrest. Breeders continuously strive to develop improved varieties by fine-tuning genetically complex yield and end-use quality parameters while maintaining stable yields and adapting the crop to regionally specific biotic and abiotic stresses.

RATIONALE: Breeding efforts are limited by insufficient knowledge and understanding of wheat biology and the molecular basis of central agronomic traits. To meet the demands of human population growth, there is an urgent need for wheat research and breeding to accelerate genetic gain as well as to increase and protect wheat yield and quality traits. In other plant and animal species, access to a fully annotated and ordered genome sequence, including regulatory sequences and genome-diversity information, has promoted the development of systematic and more time-efficient approaches for the selection and understanding of important traits. Wheat has lagged behind, primarily owing to the challenges of assembling a genome that is more than five times as large as the human genome, polyploid, and complex, containing more than 85% repetitive DNA. To provide a foundation for improvement through molecular breeding, in 2005, the International Wheat Genome Sequencing Consortium set out to deliver a high-quality annotated reference genome sequence of bread wheat.

RESULTS: An annotated reference sequence representing the hexaploid bread wheat genome in the form of 21 chromosome-like sequence assemblies has now been delivered, giving access to 107,891 high-confidence genes, including their genomic context of regulatory sequences. This assembly enabled the discovery of tissue- and developmental stage-related gene coexpression networks using a transcriptome atlas representing all stages of wheat development. The dynamics of change in complex gene families involved in environmental adaptation and end-use quality were revealed at subgenome resolution and contextualized to known agronomic single-gene or quantitative trait loci. Aspects of the future value of the annotated assembly for molecular breeding and research were exemplarily illustrated by resolving the genetic basis of a quantitative trait locus conferring resistance to abiotic stress and insect damage as well as by serving as the basis for genome editing of the flowering-time trait.

CONCLUSION: This annotated reference sequence of wheat is a resource that can now drive disruptive innovation in wheat improvement, as this community resource establishes the foundation for accelerating wheat research and application through improved understanding of wheat biology and genomics-assisted breeding. Importantly, the bioinformatics capacity developed for model-organism genomes will facilitate a better understanding of the wheat genome as a result of the high-quality chromosome-based genome assembly. By necessity, breeders work with the genome at the whole chromosome level, as each new cross involves the modification of genome-wide gene networks that control the expression of complex traits such as yield. With the annotated and ordered reference genome sequence in place, researchers and breeders can now easily access sequence-level information to precisely define the necessary changes in the genomes for breeding programs. This will be realized through the implementation of new DNA marker platforms and targeted breeding technologies, including genome editing. ■

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Read the full article at <http://dx.doi.org/10.1126/science.aar7191>

Downloaded from <http://science.sciencemag.org/> on October 13, 2019

„Wheat genome deciphered, assembled, and ordered“

- ✓ authored by more than 200 scientists from 73 research institutions in 20 countries – presents the reference genome of the bread wheat variety Chinese Spring
- ✓ precise location of 107,891 high confidence genes and of more than 4 million molecular markers
- ✓ discovery of tissue and developmental stage-related gene by transcriptome atlas representing all stages of wheat development
- ✓ complex gene families involved in environmental adaptation and end-use quality - revealed at subgenome resolution and contextualized to known agronomic single-gene or QTL
- ✓ future value of the annotated assembly for molecular breeding resolving the genetic basis of a quantitative trait locus conferring
 - ✓ resistance to abiotic stress
 - ✓ insect damage
 - ✓ basis for genome editing of the flowering-time trait



Wheat

molecular markers – intraspecies diversity

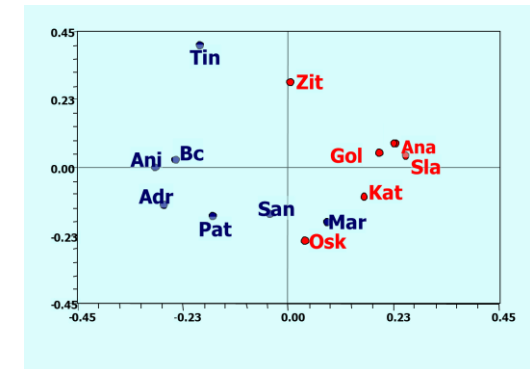
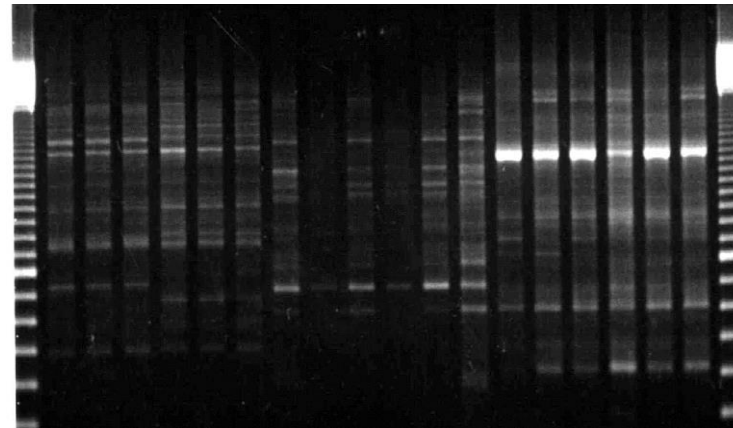
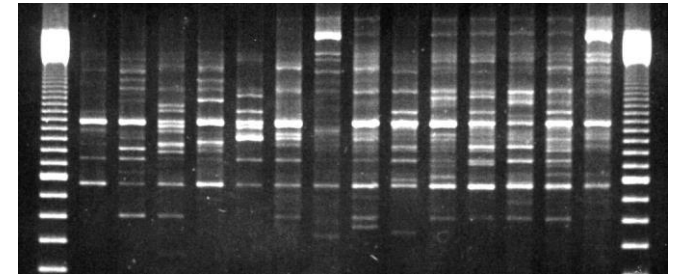
✓ advances in PCR genotyping markers have facilitated moves into marker-assisted selection in breeding programs

✓ Random Amplified Polymorphic DNAs (RAPDs)

✓ Marić (2002); Marić et al. (2004) – 14 elite wheat cultivars, 14 polymorphic markers, A total of 366 RAPD bands were amplified, among which 341 (93.2%) were polymorphic

✓ Intra-varietal diversity

✓ detection of genetic purity



Wheat

molecular markers – intraspecies diversity

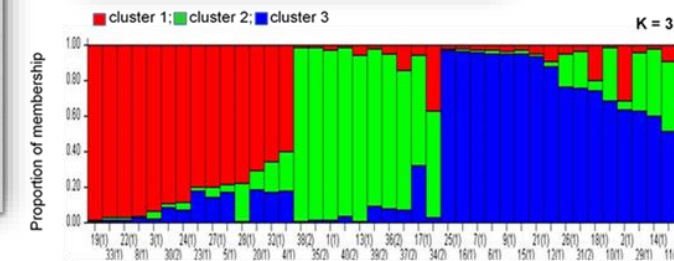
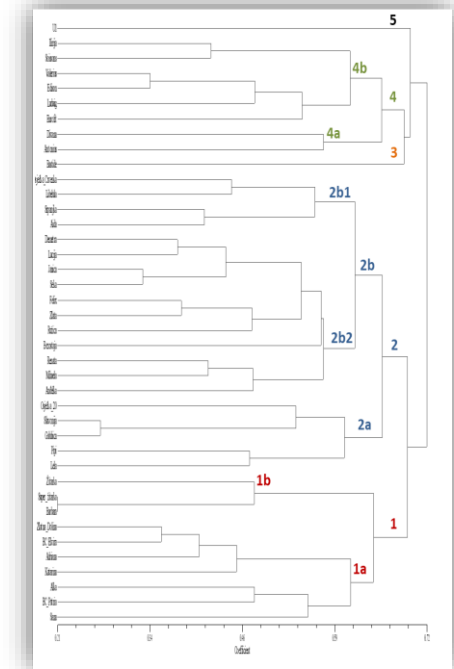
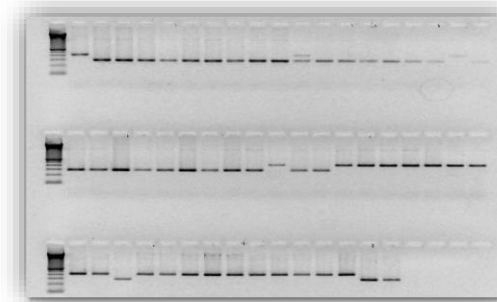
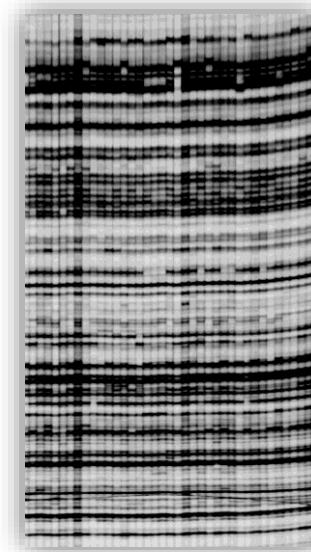
- ✓ Simple Sequence Repeats (SSRs) or microsatellites
- ✓ Dvojković (2009) - 122 cultivars (1905 – 2008), 46 SSRs, 413 different alleles, $H_e=0.69$, $PIC=0.65$, $D_{PSAM}=0.693$, 91.6%, molecular diversity between breeding programs, population structure ($K=4$)
- ✓ Dvojković et al. (2011) - allelic variability at locus *Xgwm261* (*Rht8* gene – one of the plant height genes)



Wheat

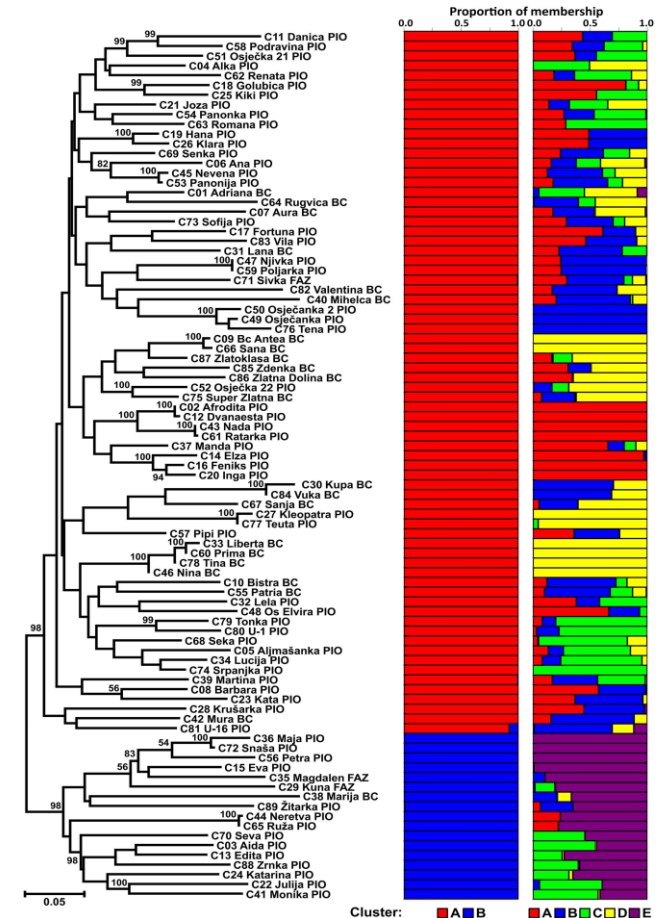
molecular markers – intraspecies diversity

- ✓ amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs)
- ✓ Petrović et al. (2011); (2012); (2018)
- ✓ 40 genotypes of different origin,
- ✓ restriction enzymes → Mse I i Sse8387I/4 primer pairs, 27 polymorphic bands, PIC=0.34; more adjusted to detect genetic relation at individual level
- ✓ 26 microsatellite pairs, 108 different alleles, H_e =from 0.255 to 0.81; more efficient in determination of population structure, grouping the varieties almost perfectly according to pedigree



Wheat molecular markers – intraspecies diversity

- ✓ Diversity Array Technology markers (DARs) - presence of a specific DNA fragment from the total genomic DNA, parallel hundreds of thousand loci in a single assay, behave in a Mendelian fashion (Wenzl *et al.*, 2004.a; Akbari *et al.*, 2006)
- ✓ Novoselović *et al.* (2016) - Croatian breeding pool (84) compared to 523 European cultivars from seven countries using 166 common DArT markers, A total of 1531 DArT markers were scored, out of which 1,229 markers were retained
- ✓ The most of the genetic diversity among cultivars within countries – geographical highly significant difference between regions 16.19% of the total variance - usefulness of open-access wheat datasets is also highlighted



Wheat

molecular markers – intraspecies diversity

- ✓ DArT markers

- ✓ Installation Research Project:

Creating Wheat for the Future – Quest for the New Genes in the Old Gene Pool (PHENOWHEAT);
Croatian Science Foundation (2014-2017)

- ✓ 364 genotypes, 5 continents, DArTs, SSR, functional markers, agro-morphological traits

- ✓ assessment of phenotypic and molecular genetic diversity of wheat breeding germplasm; identification of existing functional markers for agronomical important traits in tested germplasm; identification of marker-trait associations; outlining the most suitable parents for future crossing and breeding programs

- ✓ 11 795 retained DArT/4 507 SNP

- ✓ Guberac et al. (2017) – review paper

- ✓ SSR – DArT – morpho-agronomic traits – associative mapping – PhD thesis – January 2020

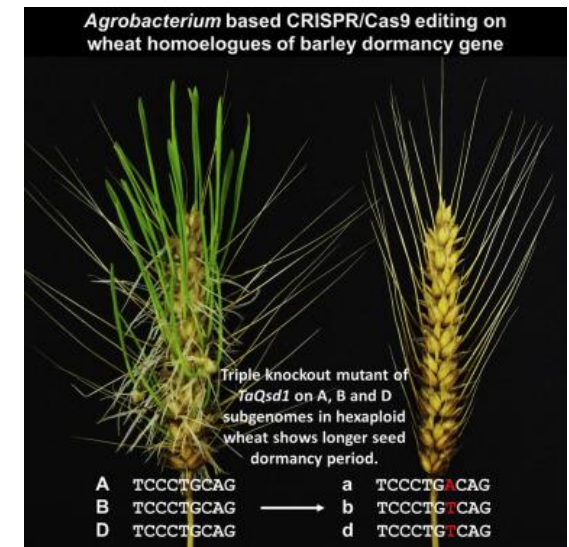


Wheat

recent and future prospects

- ✓ KASP (Kompetitive allele-specific PCR) technology sensitive method within a polyploid context (Ramirez-Gonzalez et al., 2015)
 - ✓ Pre-designed KASPs - CerealsDB and MASWheat for agronomically relevant SNPs such as grain size gene (GW2) that is being used in breeding programs (Simmonds et al., 2016)
- ✓ Wheat „Pan Genome” (2017) - genome assemblies released - modern wheat’s ancestors, wild emmer wheat (“Zavitan”) and *Aegilops tauschii*, and domesticated wheat - tetraploid wheat (“Svevo”) and hexaploid wheat (“Chinese Spring”)
- ✓ CRISPR/Cas9
 - ✓ Kim et al. (2018) - targeted editing of stress-responsive transcription factor genes, wheat dehydration responsive element binding protein 2 (TaDREB2) and wheat ethylene responsive factor 3 (TaERF3)
 - ✓ Wang et al. (2018) - The knockout mutations generated by this construct in all three homoeologous copies of one of the target genes, *TaGW2* - increase in seed size and thousand grain weight

„Genome-Edited Triple-Recessive Mutation Alters Seed Dormancy in Wheat”
Abe et al. (2019)





Sveučilište Josipa Jurja Strossmayera u Osijeku

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Thank you for your attention!

