

Crop germplasm: Molecular and physiological perspective towards achieving global crop sustainability

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Keyword: Artificial intelligence, Core-set, Climate change, Nitrogen use efficiency (NUE), Omic approaches. Plant genetic resources, Stress, System biology, Water use efficiency (WUE)

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Abstract:

Germplasm is a long-term resource management mission and investment for civilization. For both food and nutritional health, the present changing environmental scenario has become an urgent universal concern. Multiple excellent studies have been previously performed, although the advancement and innovation of practices will require the exploration of the potentiality of crop germplasm. In this study, we emphasized (i) germplasm activates, current challenges and ongoing trends of the crop germplasm, and (ii) how the system biology will be helpful to understand the complex traits such as water use efficiency (WUE), and nitrogen use efficiency (NUE) to mitigate challenges for sustainable development under growing food requirement and climate change conditions. We focused on a vision for transforming PGR into a bio-digital resource system, for the development of climate-smart crops for sustainable food production. Moreover, this review attempted to address current challenges, research gaps and describe the advanced integrated strategies that could provide a platform for future crop improvement research.

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Introduction

By introducing the *Rht* (reduced height) gene, the 60-year-old global success stories of the first green revolution (1960s) was achieved. Subsequently, the importance of crop germplasm and the conservation of crops have been recognized worldwide. To date, an estimated ~1750 plant germplasm centres globally hold 7.4 million accessions; however, <2% of these assets have been used as plant genetic resources (PGRs) (Janzen *et al.*, 2019). Recently, statistics suggested that the existing crop yield trajectory is insufficient to nourish the global population in 2050 (Bailey-Serres *et al.*, 2019). Moreover, by 2050, the global food demand will be expected to increase by 70% (Connor *et al.* 2017). Many decades later, because of global food security and increasing climate-related challenges, geneticists realized an urgent requirement for advanced approaches to comprehensively assess PGRs for potential use. Faced with these post-green revolution challenges, emerging crop breeding techniques should attempt to reach increasing requirements at a reasonable rate of crop growth. At present, the major challenge is how to exploit and capitalize on this abundant resource. In this

circumstance, for future life insurance, a vision has been proposed for transforming PGRs into a bio-digital resource system. To harness the benefit of PGRs, despite well-equipped maintenance and storage facilities, the additional execution of frontier technology transfer is lacking.

Germplasm activity, trends and current challenges

The breeding resource maintenance program is the first important component of digging into the PGRs. Figure 1 shows the structural component and activity of germplasm are divided into four major components. To harvest the benefit of PGRs, the development and maintenance of a precise “core-set” collection has been illustrated. The core-set collections should have the greatest diversity of total genetic resources (comprising both exotic and endogenous collections) because they served as a reference population for additional breeding program (Mascher *et al.*, 2019). The core-set collection should be free of genetic duplicates and must have evolutionary potential genotypes. Basic cytogenetic information such as ploidy level, mitotic behaviour, and genetic diversity/redundancy is required for an additional breakthrough. Such a core-set will be useful to understand the genetic variation in the species that can be used for innovative field experiments and subsequent parental material in the breeding program. Moreover, the cost of maintenance of germplasm could be also be reduced.

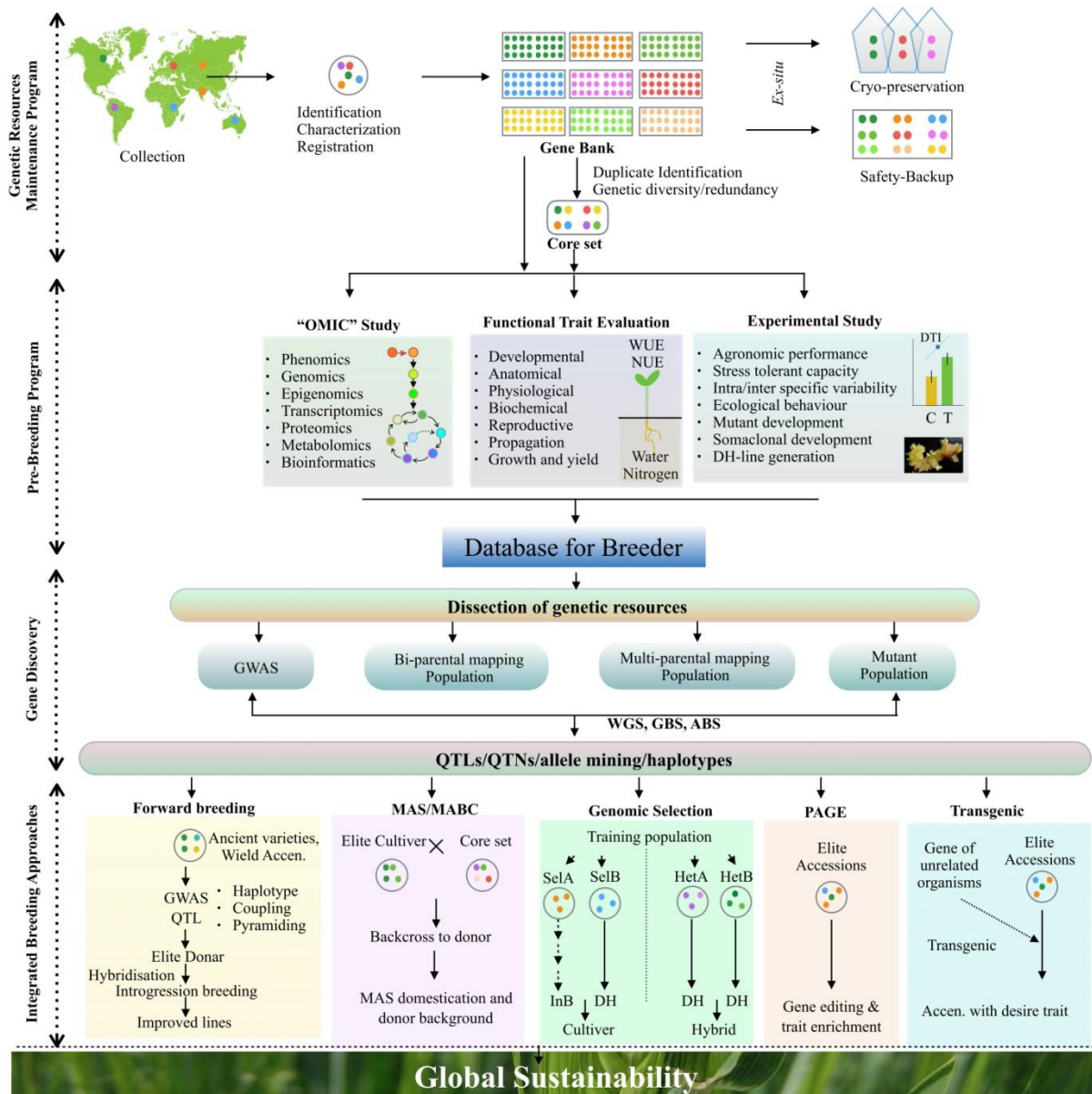


Figure 1: Structure and activity of crop improvement strategies for exploration and capitalization of genetic resources. Concerned crop improvement program divided into four major strategies. (A) *Genetic Resource Maintenance Program*: A collective model for collection to conserving breeding resources. Integrated taxonomic and cytogenetic approaches have been proposed for developing the “core-set” germplasm. A core-set collection represents a reference population of total germplasm with unique accessions. (B) *Pre-Breeding Program*: Advance pre-breeding strategy has been emphasized to reveal the potentiality of the core collection. Integrated omic approaches, functional trait evaluation, and different experimental datasets can be generating a database for plant geneticists/breeders. (C) *Gene Discovery*: For both complex traits and simple inheritance traits, the most challenging job is to harness the beneficial gene dissecting. GWAS, bi/multi-parental, and

mutation population mapping are preferably powerful strategies for establishing genetic trait association. Phenome QTL (phQTL), metabolome QTL (mQTL), proteome QTL (pQTL) and expression QTL (eQTL) have been extensively used to discover genes and functional annotation. (D) *Integrated Breeding Approaches*: Forward breeding, MAS/MABC, PAGE, and transgenic approach can be employed to integrate the simply inherited traits in which genomic selection can be employed to integrate the complex inherited traits. The aim of global sustainability will be fulfilled by this systematic evaluation of genetic resources that will simultaneously save time and budget expenditure. Abbreviation: WUE, water use efficiency; NUE, nitrogen use efficiency; DTI, drought stress index=(T/C)*100; C, control; T, treatment; GWAS, genome-wide association study; QTL, quantitative trait locus; QTN, quantitative trait nucleotide; WGS, whole-genome sequencing; GBS, genotyping by sequencing; ABS, array-based sequencing; MAS, marker-assisted selection; MABC, marker-assisted backcrossing; PAGE, promotion of alleles through genome editing; SelA/B, selfing line A/B; HetA/B, heterozygous line A/B; InB, Inbred; DH, doubled haploid.

In the context to explore genetic resources, (1) sink of genetic diversity with increase in the number of gene bank accessions; (2) accumulation of duplicate accessions over a longer period collection; (3) degradation of collection, particularly exotic accessions in response to the edaphic/biotic-abiotic factors; (4) loss of originality because of long-term domestication; (5) insufficient knowledge for characterizing complex important traits; (6) accumulation of rare deleterious alleles during crop domestication; and (7) low recombination frequency between the wild relatives have been considered the major bottleneck for crop improvement (Langridge *et al.*, 2019). Hence, clearly, new and innovative utilization strategies are required to reduce the complexity of these limitations. Therefore, for crop improvement, it is important to intelligently guide the germplasm resources. Accordingly, we should focus on a vision for transforming PGRs into a bio-digital resources system.

Currently, systems biology is considered as a potential approach for the complete understanding of biological systems (Lavarenne *et al.*, 2018). Integrated phenomics and next-generation sequencing (NGS) technologies have been utilized for identifying genetic markers associated with desirable traits in multiple crops, including rice, foxtail millet, pigeon pea, pearl millet, cotton, rapeseed, chickpea, and grape. Moreover, the large-scale re-sequencing

of germplasm accessions have been re-sequenced in rice (3010), pearl millet (994), and chickpea (429) with the ongoing and deep profound of sequencing costs (Varshney *et al.*, 2020). This study suggests that large-scale sequencing should be undertaken for all available accessions. Such projects will generate ‘big data’ that has storage and computational challenges. Therefore, the advancement of sequence-based study in next-generation breeding, high-throughput bioinformatics platforms, and passionate scientists is required. These datasets will provide the information to breeders for mining superior alleles/haplotypes that can serve as the key to select precious parental material for breeding.

Breeders mostly target functional traits, such as water use efficiency (WUE) and nitrogen use efficiency (NUE), to improve climate resilience crop productivity (Tracy *et al.*, 2020). Agriculture is the world’s largest consumer of water and accounts for 70% of all water used worldwide; by 2050, global food demand is expected to increase by 70% (Connor *et al.*, 2017). The ongoing global catastrophe of water is one of the major problems in the present-day climate scenarios and drought is considered as the most severe abiotic stress affecting crop productivity at a global level. Moreover, nitrogen is the most essential nutrient for plant’s growth, development, and yield. Increasing trends of fertilizer application to achieving productivity, resulting in the decreasing the acquisition and subsequent utilization of applied nitrogen in crop plants. Additionally, the surplus amounts of inorganic and organic nitrogen fertilizers often imposed drastic negative impacts on the environment (Kumar *et al.*, 2020). For example, NUE for cereal production is ~33% globally and the unaccountable 67% corresponds to a \$15.9 billion loss/year of nitrogen fertilizer (Raun *et al.*, 1999; Fagodiya *et al.*, 2020). Moreover, it is estimated that ~1% increase in crop NUE could annually save \$1.1 billion (Kant *et al.*, 2011; Li *et al.*, 2020). Hence, for achieving greater agricultural sustainability, the development of crop plants with drought-tolerant and more efficient nitrogen usage is, therefore, an important research challenge. Unfortunately, to date, extremely few studies has been conducted (**Table 1**).

Table 1: List of important original research on different crops and detailed information about complex traits - water use efficiency (WUE) and nitrogen use efficiency (NUE)

Complex traits	Studied Crop	Species	Number of Accession/lines studied	Condition of experiment	Associate traits studied	Approaches used	Identified gene/functions	References
WUE								
	Apple	<i>Malus domestica</i>	Two MdATG8i-overexpressing lines	Long-term drought treatment	Autophagic activity	Transgenic	<i>MdATG8i</i> -overexpressed lines exhibited higher WUE under long-term moderate drought conditions.	Jia et al., 2021
	Sorghum	<i>Sorghum bicolor</i>	659 accessions	Decrease light condition	Stomatal responses	Genomics, transcriptomics, and phenomics (High-throughput thermal imaging)	Total 77 genes related to WUE were identified. Out of those, 24 genes involved in stomatal opening/closing, 35 genes stomatal/epidermal cell development, 12 genes leaf/vasculature development, 8 genes-chlorophyll metabolism/photosynthesis.	Pignon et al., 2021
	Sorghum	<i>Sorghum sp.</i>	18 accessions	Under fluctuating light are	--	Performance-based on phenotyping	--	Pignon et al., 2021
	Cotton	<i>Gossypium spp.</i>	Two accessions and eight cultivars	Controlled condition	Root traits	Performance-based on phenotyping	--	St Aime et al., 2021
	Persian walnut	<i>Juglans regia</i>	95 walnut families	Well-watered (control) and drought	Leaf-traits	Genomics and performance-based on phenotyping	WUE traits were involved in ABA signaling.	Arab et al., 2020

stress							
Cassava	<i>Manihot esculenta</i>	High-yielding 13 farm-preferred African cultivars	Controlled condition	Photosynthetic traits	Biochemical and performance-based on phenotyping	--	De Souza et al., 2020
Rice	<i>Oryza sativa L.</i>	173 accession	Water-limited	Root traits	Marker–trait associations (MTAs)	--	Raju et al., 2016
Rice	<i>Oryza sativa L.</i>	260 introgressed lines of IR-64	water-limited	Root traits	Performance-based on phenotyping	--	Dharmappa et al., 2019
Soybean	<i>Glycine max (L.) Merr.</i>	10 genotype	Irrigation and no-irrigation	Yield, and root traits	Performance-based on phenotyping	--	Fried et al., 2019
Wheat	-	71 accessions	Water-limited	Different yield-related parameters	Performance-based on phenotyping	--	Meena et al., 2019
Soybean	<i>Glycine max L. Merr</i>	383 soybean recombinant inbred lines	Induced drought	grain yield	Genome-wide association analysis	--	Lopez et al., 2019
Wheat	<i>Triticum aestivum L.</i>	100 accessions	INA	Canopy temperature, chlorophyll content, and spot blotch (<i>Cochliobolus sativus</i>)	Performance-based on phenotyping	--	Jamil et al., 2016

resistance

NUE

Rice, Wheat, and Barley	<i>Oryza sativa</i> , cv. Nipponbare ; <i>Hordeum vulgare</i> , cv. Golden Promise; <i>Triticum aestivum</i> , cv. Gladius	<i>Agrobacterium</i> and microprojectile bombardment mediated transformed lines	Different levels of nitrogen	Growth parameters	Transgenic, transcriptomics, metabolomics	Alanine aminotransferase (<i>HvAlaAT</i>)-overexpression enhance NUE.	Tiong et al., 2021
Rice	<i>Oryza sativa L.</i>	<i>Indica</i> and <i>japonica</i> rice varieties	Different nitrogen levels	Yield traits	Genomics, transcriptomics, metabolomics, and phenomics	Quantitative trait locus <i>DULL NITROGEN RESPONSE1</i> involved NUE by auxin-mediated signaling.	Zhang et al., 2021
Eggplant	<i>Solanum melongena</i>	Four genotypes	Short- and long-term exposure to low nitrogen	Photosynthetic traits	Transcriptome	High-NUE genotypes are involved in the light-harvesting complex and receptor, a ferredoxin–NADP reductase, a catalase and WRKY33.	Mauceri et al., 2021
Pearl millet	<i>Pennisetum glaucum</i> (L.) R. Br	380 diverse pearl millet lines	Three different N levels- 0%-N0, 50%-N50, 100%-N100	Grain yield (GY)	Biochemical and performance based on phenotyping	Identified 25 nitrogen insensitive lines.	Pujarula et al., 2021

Oilseed rape	<i>Brassica napus L.</i>	Two genotypes	With or without nitrogen	Sugar concentrations	Transcriptome	A vast number of genes are associated with N-stress response and high NUE. These genes are involved in phytohormone signaling, N uptake and assimilation, amino acid metabolism, fatty acid biosynthesis, photosynthesis, C assimilation, starch biosynthesis and carbohydrate catabolism.	Yang et al., 2020
Potato	<i>Solanum tuberosum L.</i>	Three potato cultivars	N-complete group (short for N) and N-deficient nutrient group	--	Phenomics, Transcriptomics and biochemical	Nitrogen deficiency decreased the nitrate reductase (NR), glutamine synthetase (GS) and root activity.	Zhang et al., 2020
Brassica	<i>Brassica napus</i>	Two genotypes, D4-15 and D2-1 having Contrasting NUE	Control and nitrogen starvation	Root traits	Transcriptomics, metabolomics, and phenomics	A nitrate transporter gene BnaA06g04560D (<i>NRT2.1</i>) and two vacuole nitrate transporter <i>CLC</i> genes (BnaA02g11800D and BnaA02g28670D) were up-regulated by N starvation in N-efficient genotype (D4-15)	Li et al., 2020
Bermuda grass	<i>Cynodonda ctylon L.</i>	290 genotypes	High and low nitrogen	Plant biomass	--	--	Schneider-Canny et al., 2019

Wheat	<i>Triticum aestivum L.</i>	15 genotype	Low and optimum nitrogen levels	Plant biomass and grain per pot	Phenotyping	--	Nguyen et al., 2019
Rice	<i>Oryza sativa var. indica</i>	15 genotype	Low nitrogen	N-responsive germination, O ₂ -consumption, seed urease activities, root growth, crop duration, and yield	Performance-based on phenotyping and biochemical	--	Sharma et al., 2018
Rice	<i>Oryza sativa L. subsp. japonica Kato</i>	14 indices of 38 varieties	Three different nitrogen levels	Relative yield	Performance-based on phenotyping	--	Jai et al., 2010

Above discussion clearly indicates systematic approaches are still lacking to understand WUE and NUE because of (1) the complexity of polygenic regulation, (2) both traits being highly influenced by environmental factors, (3) lack of investigations at the molecular level, and (4) lack of advancement of integrated approaches. Moreover, the molecular perspective in terms of the identification of desired genes, co-expression/interaction patterns, the influence of *cis/trans*-elements, and metabolic pathways of crop WUE and NUE was not addressed well. Remarkably, when executed in the real field trial, a large number of published trait-based experiments on controlled-environment conditions did not show consistency (Rebetzke et al., 2014). At present, automated robotics phenomic platforms have been used to quantify genotypes based on functional traits such as growth form, woodiness, leaf economic spectrum, WUE, NUE, as well as stress tolerance capacity. To explore the diverse gene bank resources for developing climate resilience crops, the evaluation of agronomic performance and stress-tolerant capacity under different climatic conditions along with crop behaviour, intra/inter-specific variability, and mutant performance evaluation is necessary. Therefore, to quantify the potentiality of genotypes, next-generation trait-based evaluation should be performed on field conditions. This will help us identify superior genotypes/accessions that are stable, abundant, and environmentally less sensitive (Martin et al., 2015). For identifying the desired genes and associated traits, the recent trend of the functional phenomic approach will be helpful (Braun et al., 2020). The availability of these resources will be accelerated by increasing systems biology approaches to understand the molecular mechanism of complex traits such as WUE and/or NUE (**Figure 2**).

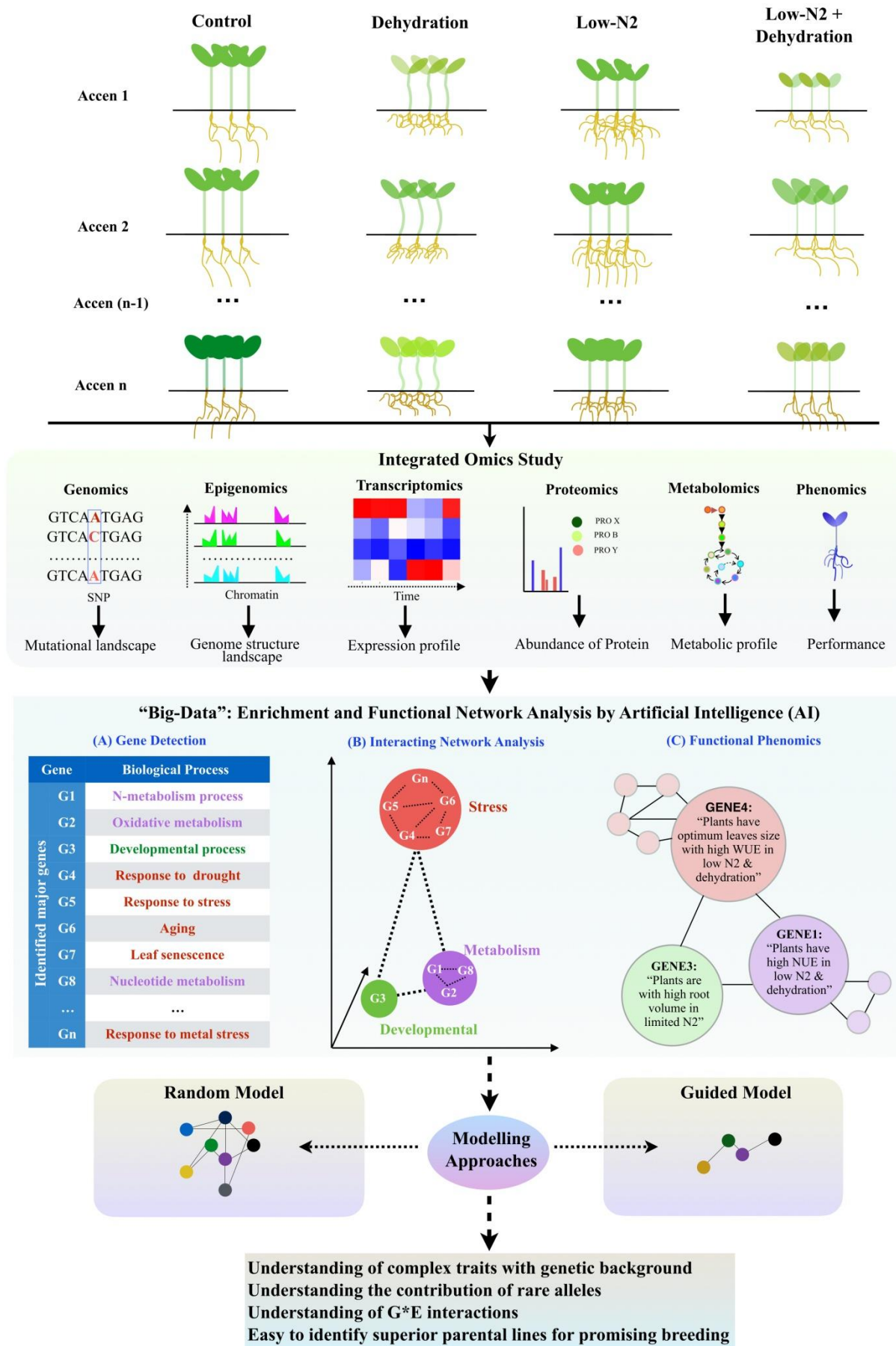


Figure 2: A comprehensive gene discovery strategy of water use efficiency (WUE) and nitrogen use efficiency (NUE) associated traits. Synthesized high-throughput multi-omic integration data will confirm the phenotypic behavior, mutational landmark (SNP), expression pattern, the abundance of protein, and metabolic signature with associated treatment. Different transcript, metabolic, protein–protein interaction libraries of different genotypes will be helpful for enrichment and functional network analysis. Previously reported different data-mining bioinformatics-based analysis, e.g., (a) NGS-based RNAseq study will be helpful to identify abundant genes, (b) multidimensional clustering and gene network analysis of proteomic study (2D gel electrophoresis and co-immunoprecipitation, etc.), and (c) functional phenomics that will help to predict and discrimination of the desired gene with the associated trait. The identification of a gene that is strongly associated with a particular phenotype, e.g., *GENE 1* strongly associated with those plants that have high NUE in low N₂ and dehydration conditions can be analyzed. Finally, big-data enrichment and functional network analysis by artificial intelligence (AI) will provide the opportunity to develop different types of models such as random models and guided models. The guided model will be more precious and accurate, which may develop as per our requirement, whereas a random model represents the overall analysis. Thus, an intelligently guided model will help in the understanding of complex traits with genetic background, G*E interactions patterns, developing ideotype breeding especially for underground traits, identify superior parental lines for promising breeding, improving smart farming.

System biology and future sustainability

To understand the genetic basis of trait variation, NGS-based genome-wide association studies (GWAS) are a powerful tool. This approach has been successfully applied to many important traits in different species, including yield-relevant traits, in crops. Sufficiently powered GWAS often identify tens to hundreds of loci containing hundreds of single-nucleotide polymorphisms (SNPs) associated with a trait of interest (McMullen et al., 2009). Once traits are correlated with specific biosynthetic/metabolic pathways and desired alleles have been identified, researchers can take up a broader understanding of crop biology to anticipate parental and allelic combinations that will discover superior agronomic traits. Although simply analyzing the genome sequences or genome-wide association study will become a “null hypothesis”, we will never get the account of gene regulation from gene/protein sequences. To date, the identification of candidate genes detected by

transcriptomics approaches and/or mapping is not available in most crops (Li et al., 2019). Furthermore, it is important to understand in-depth molecular mechanisms of their possible agronomic values. In addition to structural genomics, information on gene expression atlases, epigenome maps, proteome maps, and metabolome maps have been developed for a few crop species (Zogli et al., 2020). From the last ten years, plant molecular geneticists efficiently practice the most sophisticated toolkits for digging into valuable genetic resources that have been used such as advanced cloning strategies, genetic transformation methods, different gene-editing tools, co-immunoprecipitation, two-hybrid system, mass spectrometry, and data-mining bioinformatics. In this context, the recent study published by Jia et al. (2020) in *Nature Communications* explains that *KERNEL NUMBER PER ROW6* regulates maize grain yield, which is an outstanding example for us. Moreover, the information on the genetic architecture of important agronomical traits, their associated alleles, and the underlying molecular mechanism has remained elusive. Hence, to understand the complex biological phenomena, ongoing trends of “omic”-study will be helpful for crop improvement. For breeders/geneticists, transforming PGR into a bio-digital resource system is a prerequisite and helps us build up a database.

Germplasm is the source of novel genes. For global sustainability, selected promising genetic resources and genomic regions associated with the traits, integrated breeding approaches have been taken up. The classical paradigm of plant breeding, recurring cycles of crosses, and selections (MAS/MABC) have long been considered as a tedious method, whereas targeted genetic recombination has long been considered as a quicker method for the genetic transformation of the gene of interest from superior donors. Moreover, an array of reports suggested that plant tissue culture technology has shown considerable impact on the crop enhancement program. However, because of the lower frequency of transformation, *in vitro* recalcitrance, and different bio-safety rules, plant biotechnologists are unable to achieve the targets to date. The precise genetic engineering technology will allow the rapid transfer of advantageous traits between crops and their wild relatives in both directions. Furthermore, implementation of advanced breeding technologies including speed breeding dramatically accelerates crop development by facilitating fast generation cycles (Watson et al., 2018). Understanding the functional aspects, the conventional genetic methods-integrated strategy has the following advantages: (a) less tedious because of the multiplication of population is not required; (b) large plant sample size, mutants, and stable homozygous overexpressed plants are not required; (c) low-cost of mining of publicly available database resources makes the comparative study of omics data easier; (d) an integrated approach could identify and

provide comprehensive interactions, co-expression, and metabolic/biosynthetic grid; and finally (e) integration of advanced artificial intelligence (AI) gives the sufficient multi-dimensional data. Thus, this integrated approach should turn out to be one of the most efficient systems for a high-resolution understanding of the complex genetic mechanisms in the near future. For crop improvement, the recent trends of genome editing toolkits have significant potentiality. As a plant geneticist/breeder, our aim is to focus on the exploration and strengthen the advanced strategies to harness the benefit of ~7.4 million gene bank germplasm material. Our present brief discussion will provide the advancement of germplasm assets that will meet the required sustainability in the near future.

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