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Genetic and molecular insights from *Arabidopsis* for enhancing crop yield

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ABSTRACT

Arabidopsis is an ideal plant for research because of its tiny, fully sequenced genome and ease of genetic manipulation. Among flowering plants, *Arabidopsis thaliana* has one of the shortest genomes, measuring only 135 million base pairs which is perfect for genomic and transcriptome research because of its compact size, which allows for quicker sequencing, simpler annotation, and more effective data analysis. In the year 2000 it became the first plant to have its whole genome sequenced, which was a significant genomics milestone. Because of its unique combination of biological and practical benefits, it is used most often as a model plant in scientific studies. Genetic modification of Arabidopsis is very feasible, especially when done by the Agrobacterium tumefaciens mediated process. In addition to T-DNA insertion lines and CRISPR technology, Arabidopsis provides an unparalleled set of tools for plant genetic modification. The simple diploid genome of Arabidopsis comprises two sets of chromosomes, one from each parent. In experimental genetics, a diploid genome makes gene monitoring, mutation research and inheritance patterns easier to understand and aids in the development of more precise results. Despite its simplicity, it has a high level of genetic homology, or similarity, with many economically significant crops, including maize, wheat, and rice. This implies that knowledge gained from studying Arabidopsis can be used to comprise how genes function in other plants. The present article discusses the advantages of Arabidopsis as a model organism in genetic studies for enhancing the crop yield.

Keywords: Arabidopsis, Genetic Modification, Crop Improvement.

INTRODUCTION

A key component of contemporary agriculture is crop improvement, which aims to raise crop quality, production, and resilience in order to satisfy the expanding demands of a developing global population. Crop improvement involves a number of approaches, including genetic engineering, biotechnology, and conventional breeding methods [1].

Development of new crop varieties and hybrids with greater yield, tolerance, and adaptability is the main goal of crop improvement programs, the resistance to a range of diseases and insect pests, as well as for reducing the negative effects of abiotic pressure. In addition to pre-breeding to identify desired genes in related and wild species to extend the genetic base by transferring favorable alleles from other species, more focus is placed on the development of genomic resources for targeted traits and crops in order to achieve the intended goal [1].

In traditional breeding, various crop varieties are crossed to combine desired features including drought tolerance, disease resistance, and increased yields. Crop performance has significantly improved as a result of this centuries old technique. It may not always produce the required qualities, though, and it can be time consuming. By making it possible to transfer particular genes that produce desired features from one organism to another, biotechnology and genetic engineering have completely transformed crop improvement. Genetically modified organisms (GMOs) with enhanced characteristics including pest resistance and higher nutritional value have been created as a result of this strategy, sometimes referred to as genetic alteration. The application of cutting-edge technology, such as precision breeding and high throughput sequencing, to evaluate and choose advantageous features at the molecular level is another crucial component of crop development. With this method, scientists may breed crops more quickly and more effectively with specific modifications [1].

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Sustainable agricultural improvement also takes local community needs, biodiversity preservation, and the environment into account. Researchers may create crops that are not only economically viable and environmentally friendly, but also productive, by utilizing a variety of genetic resources and eco-friendly techniques. Plant development has impacted crop domestication and breeding. Only a few genes separate cauliflower, popcorn, and kale from progenitor plants that modern farmers would only consider weeds. Since flowering plants represent a relatively new evolutionary group, Arabidopsis has been used as a model to identify many of the genes involved [1].

More generally, multicellular development occurred independently in each kingdom as plants and animals split off from a common but unicellular progenitor. As a result, we observe that while specific molecules are rarely conserved, fundamental basic principles such as the crucial role of transcription factors and signaling hierarchy (hormones, receptors, and peptides) are acknowledged and found in every kingdom.

But these are just broadly similar (perhaps except for animal and plant steroids). In reality, macromolecules like mRNA, short RNA, and transcription factors themselves can move directly between cells in plants' highly interconnected supracellular vascular system, whereas this has only infrequently been seen in mammals.

Therefore, common developmental pathways probably had a purpose in unicellular or oligocellular predecessors and might have been appropriated to perform comparable tasks on their own. Instead of multicellular transcriptional memory, conserved epigenetic processes, including the Polycomb system, may have played roles in chromosome biology, genome organization, genome defense, and cellular differentiation in the ancient unicellular eukaryote. The identification of these conserved functions both within and between kingdoms is largely being aided by Arabidopsis. molecular biology [1].

LIFE CYCLE OF ARABIDOPSIS

Both haploid and diploid life cycle phases named for the spores they produce are present in all plants. The diploid phase is known as the "sporophyte" (spore bearing plant) because it facilitates meiosis to produce spores. When these haploid spores germinate, they give rise to male and female gametophyte. The gametes combine to create diploid zygotes during fertilization. Mosses spend most of their time in the gametophyte phase, ferns lie somewhere in between, while Arabidopsis and other higher plants spend their time as sporophytes [2]. The gametophytic phase in flowering plants lasts only two or three mitotic divisions, and unlike in animals, the germ line emerges from blooms on the adult plant rather than being contained in the embryo.

Arabidopsis is a hermaphrodite plant, producing haploid gametophytes of both sexes from floral parts or differentiated flowers where meiosis takes place in both male and female. However, some plants are dioecious and, though they are uncommon, can even have differentiated sex chromosomes. After fertilization, embryogenesis, and dormancy, Arabidopsis undergoes further stages that result but unlike animals, these extra embryonic tissues also known as the endosperm are the product of independent fertilization between a second haploid sperm cell and the female's diploid "central" cell, which is formed by the fusing of two haploid egg cell sisters [2].

The triploid nucleus divides quickly, followed by cellularization and the accumulation of proteins and carbohydrates, which give the embryo vital nutrients. In many plants, including Arabidopsis, the endosperm is ephemeral and is consumed by the developing embryo. However, in other plants, it can endure until germination and provide starch for staple crops like wheat and rice [2]. *Arabidopsis thaliana* is the tiny flowering plant and is a member of the Brassicaceae family. It is indigenous to Europe, Asia, and some regions of North Africa and typically grows in open, disturbed spaces like fields, rocky slopes, and roadside ditches. In genetics and plant biology, *A. thaliana*, a winter annual with a brief life cycle, is frequently used as a model organism. At roughly 135 megabase pairs, the genome of *A. thaliana* is very small for a well-developed multicellular eukaryote and is a useful means for understanding the molecular biology of several plant attributes, including light sensing and flower production, as it was the first plant to have its genome sequenced. It produces a lot of seeds, grows readily in tiny spaces with little care, and has a short life cycle of only 6 to 8 [2,3].

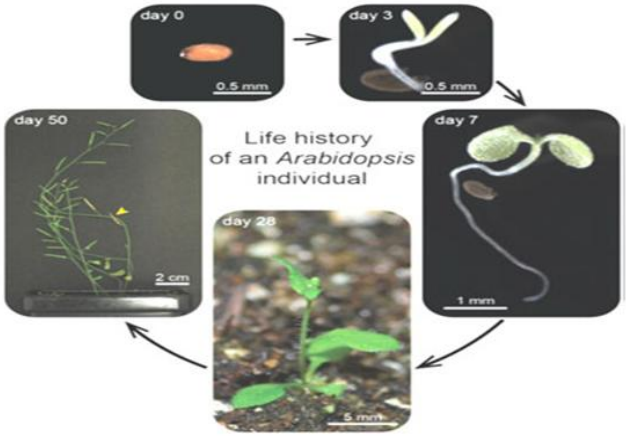

Figure 1: Genome sequence of *A. thaliana* (adopted from Makki RM *et al* [3])

GENETIC FRAMEWORK OF ARABIDOPSIS

Only 105 Mb of euchromatic DNA, roughly 15 Mb of sequenced heterochromatin, plus an additional 15-25 Mb of satellite repeats and rDNA make up the Arabidopsis genome, which is roughly 140 Mb. Each of the five centromeres is flanked by the majority of the sequenced heterochromatin, while chromosomal arms contain smaller heterochromatin areas known as knobs. It was possible to sequence 99 percent of the 29,000 Arabidopsis genes by sequencing the euchromatic portion and a large portion of the heterochromatin. Unlike many other larger plant genomes, which have much longer intergenic areas, these genes are just roughly 4.6 kilobases apart. While many animal genes have extensive regulatory sequences (up to 10 kb), tiny intergenic regions also imply small regulatory sequences for the genes. One characteristic of Arabidopsis genes that sets them apart is the GC richness of their exons (44%), which is higher than the GC content of their introns (32%) [4]. Due to the fact that the last duplication occurred only a few million years ago, approximately 25% of Arabidopsis genes still have a functional homolog, which causes significant genetic redundancy and makes reverse genetic strategies more difficult. However, this redundancy may have contributed to the success of forward genetics in Arabidopsis by enabling the use of high doses of mutagens (e.g., irradiation, ethyl nitrosourea, or ethyl methane sulphate) without causing plant death, allowing a relatively small number of mutagenized seed to reach saturation. By simply allowing the seeds to germinate and self-pollinate, it is possible to directly mutagenize seeds and recover recessive mutations. Positional cloning of mutations discovered by forward genetics is fairly easy since the genome sequence and several polymorphic strains are available. Tilling, a reverse genetics technique that involves screening DNA from mutagenized plants for point mutations in desired genes, is another application for EMS mutagenesis [2].

This approach is expected to become even more useful with the advancement of very high sequencing technologies, which are able to recover the entire range of allelic variation in every gene. The availability of the genome sequence has enabled numerous other genomic technologies, including proteomic technologies, high throughput protein localization, and tiling microarrays [2, 4].

COMPARISON OF ARABIDOPSIS GENES WITH ANIMAL GENES

Comparing Arabidopsis gene content to that of the multicellular animal genomes that were sequenced at the time, it was discovered that there were roughly 11,500 singletons and 15,000 distinct gene families in Arabidopsis, which was slightly more than that predicted for the nematode worm, C. elegans (13,601) and slightly less than that predicted for the fruit fly, D. melanogaster (18,424). Nonetheless, a large number of genes (likely housekeeping genes) were shared by plants and animals, indicating that they shared a common ancestor. The genome of Arabidopsis also contained genes that were specific to plants, such as those that were involved in the creation and alteration of cell walls and water transporting channels [2].

In green plants like Arabidopsis, 25% of the genes encode signal peptides used in intracellular transport, compared to 5% in animals and fungi, where signal peptides are primarily needed. Other genes in plants encode for signal peptides that direct transport of specific nuclear encoded proteins to chloroplasts [2].

ARABIDOPSIS IN FORWARD GENETICS

Because of its distinct biological and genomic advantages, *A. thaliana* has emerged as the most popular plant model for forward genetic research. Finding the gene causing a mutant trait is the first step in forward genetics. Arabidopsis is especially well suited for this kind of phenotype to gene investigation due to its life history and experimental viability [2,5,6]. Genetic diversity is originally produced by mutations. Chemical mutagens, particularly ethyl methanesulfonate (EMS), which causes point mutations throughout the genome, are most frequently used to induce mutagenesis in Arabidopsis. Additionally, insertional mutagens like T-DNA and transposons, as well as physical mutagens like fast neutron radiation, have been extensively used [2,7]. Large populations of plants are examined for phenotypic problems following mutagenesis. Developmental characteristics, blooming time, dwarfism, light responses, hormone modulation, and resistance to biotic and abiotic stress are examples of these phenotypes. The next step after identifying a mutant of interest is to map the altered gene and ascertain how the condition is inherited. In the beginning, recombination-based mapping with molecular markers was the mainstay of classical forward genetics in Arabidopsis. Later, researchers were able to precisely pinpoint mutant loci due to map based cloning techniques [8]. Whole genome sequencing has expedited gene identification and shortened the time required to connect phenotypes to molecular alterations because of advances in sequencing technology.

Finding biological pathways and gene functions in Arabidopsis has been made possible due to forward genetics. Studies on floral development made one of the most significant contributions. The famous ABC model of flower formation was created as a result of the isolation of floral homeotic mutants like apetala 1, apetala 2, pistillata, and agamous [9]. Similar to this, studies on light signaling mutants like hy, phy, and cop revealed important Heme regulators and showed how photomorphogenesis plants convert light signals into developmental reactions [10]. Plant hormone signaling was also made clear by forward-genetic research. For instance, the identification of hormone receptor components and downstream transcriptional regulators was made possible by mutants like ein (ethylene insensitive), gal (gibberellin insensitive), and axr (auxin resistant) [11]. These findings have far reached effects that go well beyond Arabidopsis. Enhanced stress tolerance, optimal flowering time, and improved disease resistance are just a few of the crop enhancement templates made possible by genes and pathways discovered by forward genetics. Arabidopsis is used as a translational model for agriculturally significant crops because many regulatory genes are conserved among plant species.

ARABIDOPSIS IN REVERSE GENETICS

Reverse genetics has uncovered genes for blooming time, hormone routes, stress responses, photoreception, and metabolic processes. For example, knockout studies helped uncover components of the auxin signaling system, such as TIR1 and Aux/IAA proteins [12]. By integrating mutant phenotypes with transcriptomics, metabolomics, and protein interactions, high throughput resources enable a deeper knowledge of systems.

Crop improvement techniques, such as altering flowering time, improving stress tolerance, or maximizing nutrient usage, are informed by lessons learned from Arabidopsis reverse genetics [13].

T-DNA insertional mutagenesis is the most extensively utilized reverse genetics approach in Arabidopsis. A section of DNA (T-DNA) from the Ti plasmid randomly integrates within the plant genome during Agrobacterium-mediated transformation, upsetting genes or regulatory areas. By mapping the genome, regulatory region genes are disrupted. Researchers can deduce gene function from phenotypic implications by locating insertion sites and describing homozygous mutant strains [13].

Hundreds of thousands of distinct T-DNA insertion lines have been produced by large scale collections such as SALK, SAIL, and GABI-Kat. ABRC (Arabidopsis Biological Resource Centre) and the NASC (Nottingham Arabidopsis Stock Centre) make these resources accessible to the general public. For example, a T-DNA construct with an easily amplifiable left border was used to create SALK lines, which made genotyping simple. T-DNA insertion lines make it possible to identify knockout mutants in large quantities. Researchers can choose insertions that interfere with various functional domains or validate findings because many genes have several insertion alleles. Additionally, reporter or selectable marker cassettes are commonly included in T-DNA lines, enabling expression investigations or gene

Mobile DNA elements like Ac/Ds (Activator/Dissociation) or En/Spm are used in transposon-based mutagenesis to produce insertional mutations. Transposons, in contrast to T-DNA, are able to travel throughout the genome and produce germline or somatic insertions [14]. Transposition frequency varies and frequently necessitates intricate genetic crosses. Transposon lines are less standardized and accessible in lesser quantities than T-DNA insertions.

RNA INTERFERENCE AND GENE SILENCING

Arabidopsis has adopted RNA interference (RNAi) to silence genes by producing double-stranded RNA, which is then processed into small interfering RNAs (siRNAs). These siRNAs direct the target mRNA's translation suppression or destruction [15]. The efficacy of silencing was greatly increased with the creation of hairpin RNA constructs (hpRNA) where null mutants are fatal; RNAI allows important genes to be knocked down. Through sequence-targeting design, it also permits the simultaneous silencing of several members of the gene family. Transgenic lines may have different silencing efficiencies, and insufficient knockdown can make it difficult to interpret phenotypes. Targeting conserved domains can potentially result in off-target consequences.

Artificial microRNAs exploit the plant's endogenous microRNA processing machinery. Using the backbone of a known microRNA precursor, researchers replace the native miRNA sequence with a designed 21-nt sequence that targets a specific gene [16]. Transgenic lines may have different silencing efficiencies, and insufficient knockdown can make it difficult to interpret phenotypes. Targeting conserved domains can potentially result in off-target consequences.

TARGETED GENOME MODIFICATION TECHNOLOGIES

Zinc finger nucleases (ZFNs) and transcription activator like effector nucleases (TALENS) were used to try targeted gene editing in Arabidopsis prior to the widespread use of CRISPR. These systems were labour intensive and technically challenging, but they depended on designed protein-DNA interactions [17].

CRISPR/Cas GENOME EDITING

Reverse genetics in Arabidopsis was transformed by CRISPR/Cas9 and related systems. The Cas nuclease is guided to a particular genomic sequence by a guide RNA (gRNA), causing double-strand breaks that are fixed by homologous recombination or non-homologous end joining [18].

PAM sequences, which limit targetable locations, are necessary for CRISPR systems. Despite being very low in plants, off-target action is still a risk. Depending on the tissue or developmental stage, efficiency can change. Reverse genetics investigations are made easier by inducible systems, amiRNA design software (e.g., WMD3), CRISPR toolkits, and gateway-compatible vectors. Reverse genetics investigations are made easier by inducible systems, amiRNA design software (e.g., WMD3), CRISPR toolkits, and gateway-compatible vectors.

ROLE OF ARABIDOPSIS IN DEVELOPMENT AND PATTERN FORMATION

Embryogenesis and Plant Body Formation

Knowledge of early embryonic pattern formation has significantly increased because of research on Arabidopsis. Many mutations that interfere with the formation of radial and apical-basal axes have been found through genetic investigations. For example, the gnome mutant showed that the establishment of embryonic polarity depends on vesicle trafficking [19]. Auxin signaling has a key role in defining the basal region of the embryo, specifically the establishment of the root meristem, as demonstrated by the monopteros (mp) mutant [20].

These findings have explained how the fundamental plant body plan is produced by the concerted action of transcription factors, hormone gradients, and cellular communication. Arabidopsis studies continue to be the gold standard for research on embryogenic development since early embryos of many crop plants are more difficult to alter.

Shoot and Root Meristem Development

Plant development is largely dependent on the growth and upkeep of meristems, which are areas of constant stem cell activity. Arabidopsis has a particularly well characterized shoot apical meristem (SAM), where stem cell homeostasis is regulated by a feedback loop including the WUSCHEL (WUS) and CLAVATA (CLV) genes [21]. While CLV signaling limits WUS expression to stop unchecked meristem proliferation, WUS facilitates stem cell identity in the central zone. For plant stem cell niches, this regulatory architecture has emerged as a standard paradigm.

Arabidopsis research revealed the functions of the transcription factors SCARECROW (SCR) and SHORT-ROOT (SHR) in radial patterning and cell-type specification in the root apical meristem (RAM) [22,23]. Arabidopsis roots' transparency and accessibility have allowed for the tracking of individual cell lineages throughout development, yielding hitherto unheard-of information regarding root architecture and tissue organization.

Hormone Signaling and Developmental Patterning

The study of plant hormone signaling has perhaps profited more from Arabidopsis research than any other area of plant growth. Arabidopsis mutants and reporter lines have been used to analyze auxin, cytokinin, gibberellin, ethylene, and brassinosteroids at the molecular level. Following the identification of the PIN-FORMED (PIN) family of auxin efflux carriers, which control the creation of auxin gradients essential for organ initiation, vascular development, and tropic responses, auxin in particular gained attention [24,25]. Similar to this, the discovery of DELLA proteins in gibberellin signaling shed light on hormone crosstalk and growth suppression [26].

Flower and Reproductive Development

Clarifying the genetic regulation of floral organ specification is one of Arabidopsis's most significant contributions to developmental biology. The ABC model, which specifies sepals, petals, stamens, and carpels through combinations of A, B, and C class genes, was developed based on research on Arabidopsis and Antirrhinum majus [27]. Since then, the ABC model has evolved into the ABCE and ABCDE models, adding new gene families like SEPALLATA (SEP), which are involved in meristem termination and floral organ identification. These findings have wide-ranging effects on floral morphology, reproductive development, and crop breeding [27].

Light Signaling and Photomorphogenesis

Light signaling is another fundamental aspect of Arabidopsis study. Plants' perception of red, far-red, and blue light was disclosed by mutants lacking photoreceptors such as phytochromes and cryptochromes. Molecular mechanisms that control seedling development in light vs dark settings were discovered by genetic screens that identified constitutive photomorphogenesis (cop) and de-etiolated (det) mutants [28]. These pathways provide a complete picture of how light signals interact with developmental programs by influencing blooming time, hypocotyl elongation, and chloroplast formation.

FUTURE POTENTIAL OF ARABIDOPSIS BASED STUDIES

In the field of plant research, *A. thaliana* remains a potent source of innovation. It is anticipated that technology will only increase in value as it develops, particularly in fields related to climate adaption, agriculture, and sustainability. As systems biology and AI-assisted data analysis gain popularity, Arabidopsis provides a rich yet manageable framework for simulating intricate gene regulation networks. By simulating complex biological systems, Arabidopsis enables researchers to forecast how plants will react to genetic or environmental changes. It is being used by researchers to create novel metabolic pathways, modify gene circuits, and even develop artificial features like enhanced photosynthesis or food absorption. Future crops may become more intelligent and productive as a result of these developments.

CONCLUSION

Much significant advancement in plant science have been based on *A. thaliana*, which provides the basis for knowledge about how plants develop, react to their surroundings, and protect themselves. The knowledge gained from Arabidopsis continues to speed up crop trait engineering and gene discovery, promoting climate resilience and global food security. Arabidopsis has life cycle and genomic organization characteristics that make it amenable to several genetic study techniques. It has the characteristics that can be used both in molecular genetics and some are significant for classical genetics.

Conflict of interest

The authors declared no conflict of interest.

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