



智慧化與基因科技在加速育種的應用： 從分子設計到精準選育

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摘要

因應氣候變遷的挑戰，作物育種必須朝向具備氣候韌性的特性發展，例如耐熱、耐旱與耐澇等逆境能力。同時，邁向淨零排放與減碳的農業生產模式，亦使得低甲烷排放與高氮效率品種成為新的育種目標。此外，水稻與雜糧在加工與加值應用上的需求不斷增加，也使得育種目標更為多元化。

在此背景下，如何善用最新科技，以精準方式產生變異與進行選拔，成為提升育種效率、加速新品種育成的關鍵課題。近年來分子標誌輔助選拔技術日趨成熟，基因體選拔、基因編輯以及人工智慧(AI)亦快速發展，這些先進工具能有效提高基因變異創造與目標性狀選拔的精準度，協助育種者在更短的時間內獲得優異品系。表型性狀受到基因與環境共同影響，而遺傳力的解析是育種的核心問題之一。過去數十年的研究累積了大量基因功能與調控資訊，可藉助如 ChatGPT 等大型語言模型快速搜尋與彙整相關知識，再利用 Notebook LLM 閱讀研究論文，選定候選基因後，進一步以基因編輯或分子標誌輔助選育驗證與應用。然而，在氣候劇變與環境壓力日益不確定下，仍需積極從種原中探勘新基因，特別是近緣野生種所蘊藏的抗逆境遺傳資源。若能結合基因體、轉錄體、代謝體與表型體等全方位的體學資料，將能更有效且快速地發掘新品質與抗逆基因。多數重要農藝性狀，如成熟期、株高、耐非生物逆境、品質與產量等，皆屬典型複雜量性狀，受多基因調控，並受到基因交互作用與基因 × 環境交互作用影響，遺傳力往往偏低。在此情況下，若能整合多體學資料，以統計回歸模型、深度學習或機器學習方法進行基因體選拔與數位育種，將大幅提升選拔效率與精準度。

綜上所述，當代育種已不再僅是傳統農學的延伸，而是結合農園藝、基礎植物科學、生物資訊與資料科學等多領域的跨學科工作。唯有跨領域人才的培育與跨團隊合作的深化，方能充分提升作物育種的潛能，因應未來農業面臨的全球性挑戰。

關鍵字：種原、基因編輯、分子標誌輔助選育、多體學、數位育種、基因體選拔、人工智慧、影像資料分析

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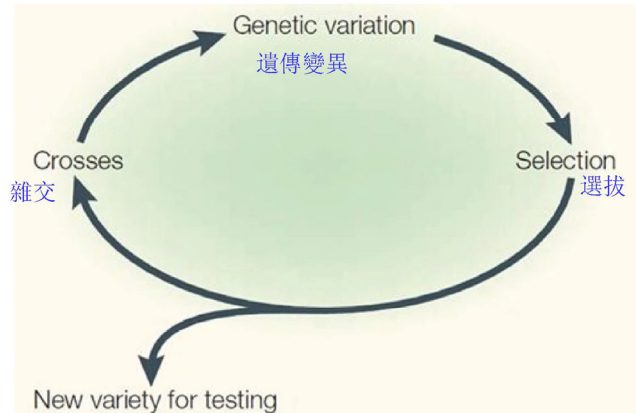
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育種—中心條例



二大要素

- 遺傳變異的來源
- 選拔效率

Figure 1 | **The central dogma of plant breeding.** The crop improvement cycle is repeated for several generations until the breeder is satisfied with the product and releases a new variety for testing. In modern agriculture, hundreds of varieties are released each year and very few of these end up in the market place.

Dani Zamir (2001); Nature Rev Gen 2: 983-989



育種

✿ 遺傳變異

➤ 基因改造

✓ Genetic modified organism, GMO

➤ 基因編輯

✓ Gene editing, GE

✓ Genome editing, GE

✿ 選拔

➤ 分子標誌輔助育種

✓ Marker-assisted breeding, MAS

➤ 基因體選拔

✓ Genomic Selection, GS

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善用基因研究以提升作物育種效率

性狀 (V_p) = 基因 (V_g) + 環境 (V_e)

遺傳力 (h^2) = 基因 (V_g) / 性狀 (V_p)

✿ 基因編輯

➤ 特定基因產生變異

✿ 分子標誌輔助選育

➤ 提升選拔效率

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Genome Editing 基因編輯



The Nobel Prize in Chemistry 2020

The Royal Swedish Academy of Sciences has decided to award the Nobel Prize in Chemistry 2020 to

Emmanuelle Charpentier

Max Planck Unit for the Science of Pathogens, Berlin, Germany

Jennifer A. Doudna

University of California, Berkeley, USA

"for the development of a method for genome editing"

Genetic scissors: a tool for rewriting the code of life

- In plant breeding, researchers can give plants specific characteristics, such as the ability to withstand drought in a warmer climate.



CRISPR-Cas9的核心機制涉及化學反應，特別是DNA的切割、修復和修飾。

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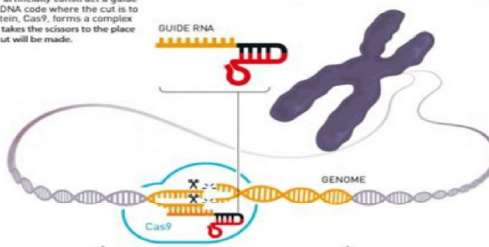


遺傳的剪刀

The CRISPR/Cas9 genetic scissors

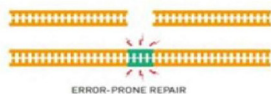
When researchers are going to edit a genome using the genetic scissors, they artificially construct a guide RNA, which matches the DNA code where the cut is to be made. The scissor protein, Cas9, forms a complex with the guide RNA, which takes the scissors to the place in the genome where the cut will be made.

Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated proteins



A 非同源性末端結合

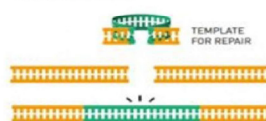
Researchers can allow the cell itself to repair the cut in the DNA. In most cases, this leads to the gene's function being turned off.



ERROR-PRONE REPAIR

B 同源性重組

If the researchers want to insert, repair or edit a gene, they can specially design a small DNA template for this. The cell will use the template when it repairs the cut in the genome, so the code in the genome is changed.



INSERTED DNA

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基因編輯 Gene Editing

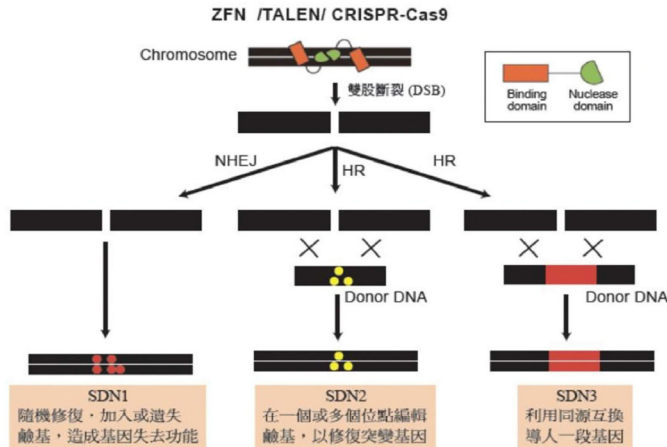


圖1. ZFN、TALEN、CRISPR/Cas9等三種基因編輯方法修補斷裂DNA機制。可客製化定點斷裂的DNA經過修補後，產生鹼基缺失、表達框移位或嵌入一段相類似的DNA，最終造成基因功能喪失、基因序列修復、或基因嵌入。SDN (Site-directed nuclease) 表示標的序列專一性核酸酶，NHEJ (Non-homologous end joining) 表示非同源互換端點黏合系統，HR (Homologous recombination) 表示雙股同源互換DNA修補系統修復。

洪傳揚 (2018)作物、環境與生物資訊 15:101-115

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基因編輯的效力

Figure 7 – The types of changes that CRISPR and similar genome editing tools can introduce in the DNA

The unedited sequence	CGTAGTCCGTGGATCGGATCGTTGACAACCTCGAA
Delete a few letters of DNA	CGTAGTCCGTGGAT ... TCGTTGACAACCTCGAA
Insert a few DNA letters	CGTAGTCCGTGGATCGG TG ATCGTTGACAACCTCGAA
Change one or a few letters	CGTAGTCCGTGGAT GG ATCGTTGACAACCTCGAA
Replace one gene with other version	C TTAGTCCGT C GATCGGAT C GAAGACAACCTCGAA
Insert a complete gene at desired location	CGTAGTCCGTGG – gene X – ATCGGATCGTTGACAACCTCGAA

EPRS | European Parliamentary Research Service (2022)

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基因編輯的效力

Figure 8 – Genome editing enables targeting multiple copies of a gene at the same time

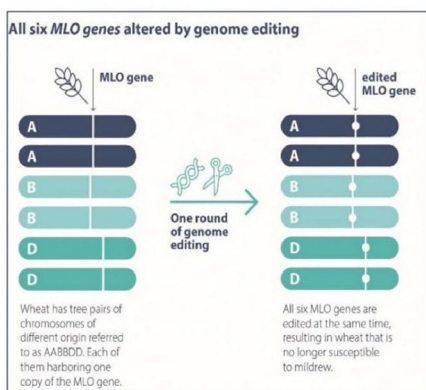
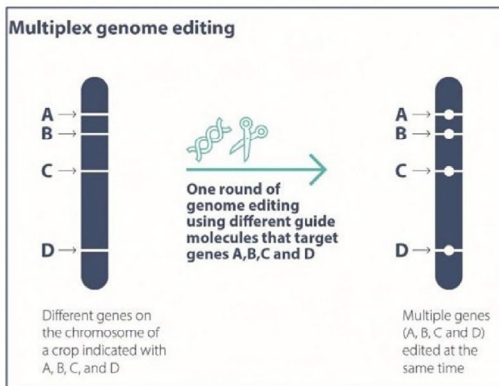


Figure 9 – Genome editing enables targeting genes at multiple locations at the same time



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基因編輯



European Sustainable Agriculture Through Genome Editing <https://www.eu-sage.eu/index.php/>

Genome-editing techniques are promising tools in plant breeding. To facilitate a more comprehensive understanding of the use of genome editing, EU-SAGE developed an interactive, publicly accessible online database of genome-edited crop plants as described in peer-reviewed scientific publications. The aim of the database is to inform interested stakeholder communities in a transparent manner about the latest evidence about the use of genome editing in crop plants. Different elements including the plant species, traits, techniques, and applications can be filtered in this database.

PLANT	TRAITS CATEGORIES	COUNTRIES	GENOME EDITING TECHNIQUE
<input type="checkbox"/> Rice (<i>Oryza sativa</i>) (278) <input type="checkbox"/> Tomato (<i>Solanum lycopersicum</i>) (125) <input type="checkbox"/> Maize (<i>Zea mays</i>) (85) <input type="checkbox"/> Soybean (<i>Glycine max</i>) (55) <input type="checkbox"/> Wheat (<i>Triticum aestivum</i>) (48) <input type="checkbox"/> Canola (<i>Brassica napus</i>) (38) <input type="checkbox"/> Potato (<i>Solanum tuberosum</i>) (35) <input type="checkbox"/> Tobacco (<i>Nicotiana tabacum</i>) (23) <input type="checkbox"/> Barley (<i>Hordeum vulgare</i>) (38) <input type="checkbox"/> Pepper (<i>Peperum</i>) (12) <input type="checkbox"/> Cucumber (<i>Cucumis sativus</i>) (11) <input type="checkbox"/> Watermelon (<i>Citrullus lanatus</i>) (10) <input type="checkbox"/> Grapevine (<i>Vitis vinifera</i>) (9) <input type="checkbox"/> Cassava (<i>Manihot esculenta</i>) (9) <input type="checkbox"/> Cotton (<i>Gossypium hirsutum</i>) (8) <input type="checkbox"/> Banana (<i>Musa spp.</i>) (8) <input type="checkbox"/> Lettuce (<i>Lactuca sativa</i>) (7) <input type="checkbox"/> Chinese cabbage (<i>Brassica rapa</i>) (7) <input type="checkbox"/> Camellia (<i>Camelina sativa</i>) (7) <input type="checkbox"/> Sorghum (<i>Sorghum bicolor</i>) (6) <input type="checkbox"/> Wild strawberry (<i>Fragaria vesca</i>) (5) <input type="checkbox"/> Benth (<i>Nicotiana glauca</i>) (5)	<input type="checkbox"/> Traits related to biotic stress tolerance (175) <input type="checkbox"/> Traits related to abiotic stress tolerance (80) <input type="checkbox"/> Traits related to improved food/feed quality (185) <input type="checkbox"/> Traits related to increased plant yield and growth (195) <input type="checkbox"/> Traits related to industrial utilization (112) <input type="checkbox"/> Traits related to herbicide tolerance (58) <input type="checkbox"/> Traits related to product color/flavour (53) <input type="checkbox"/> Traits related to storage performance (24)	<input type="checkbox"/> China (146) <input type="checkbox"/> USA (714) <input type="checkbox"/> South Korea (45) <input type="checkbox"/> Japan (42) <input type="checkbox"/> France (33) <input type="checkbox"/> UK (31) <input type="checkbox"/> Germany (30) <input type="checkbox"/> India (28) <input type="checkbox"/> Australia (28) <input type="checkbox"/> Pakistan (15) <input type="checkbox"/> Italy (15) <input type="checkbox"/> Israel (15) <input type="checkbox"/> Egypt (13) <input type="checkbox"/> The Netherlands (12) <input type="checkbox"/> Belgium (12) <input type="checkbox"/> Saudi Arabia (10) <input type="checkbox"/> Canada (9) <input type="checkbox"/> Vietnam (8) <input type="checkbox"/> Turkey (7) <input type="checkbox"/> Spain (7) <input type="checkbox"/> Korea (7) <input type="checkbox"/> Philippines (6) <input type="checkbox"/> Czech Republic (6)	<input type="checkbox"/> CRISPR/Cas (808) <input type="checkbox"/> TALENs (30) <input type="checkbox"/> ZFN (28) <input type="checkbox"/> ZFN (7) <input type="checkbox"/> ODM (6) <input type="checkbox"/> PE (4)
<p>Displaying 1000 results on Oct 30, 2025</p> <p>Displaying 904 results on June 04, 2024</p> <p>Displaying 882 results on April 26, 2024</p> <p>Displaying 850 results on Jan 26, 2024</p>			<p>SDN TYPE</p> <input type="checkbox"/> SDN1 (808) <input type="checkbox"/> SDN2 (24) <input type="checkbox"/> SDN3 (6)

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基因編輯在作物育種之應用

Banana, removal of banana streak virus	Early flowering rice
Camelina with altered oil composition	Carotenoid enriched rice
Fungal resistant grapevine	Soybean with increased oil and protein content
Waxy maize hybrid	Strawberries that flower multiple times
Maize with enhanced grain yield	Sugarcane adjusted saccharification behaviour
Maize with enhanced yield under drought stress	Tomato, self-pruning, early flowering
Non-browning mushroom	Tomato, improved shelf life
Mustard with improved flavour	High lycopene tomato
Amylopectin potato	High GABA tomato
Potato with no glycoalkaloids	Reduced allergens in wheat
Peanut with altered oil composition	High fibre wheat
Rice with enhanced grain size and number	Low gluten wheat
Rice with disease resistance	Fungal resistant wheat

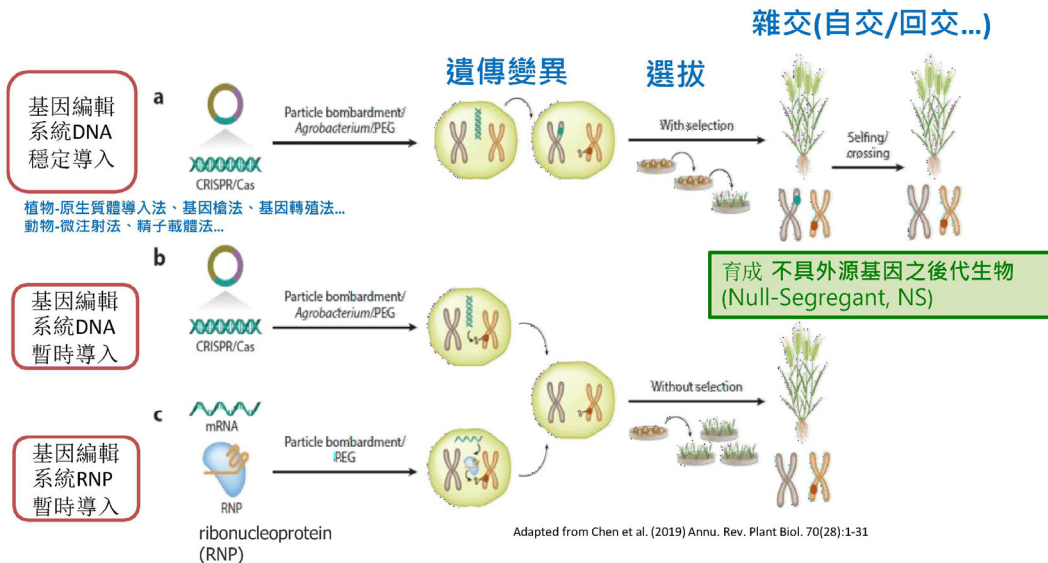
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基因編輯-育種過程及終產物



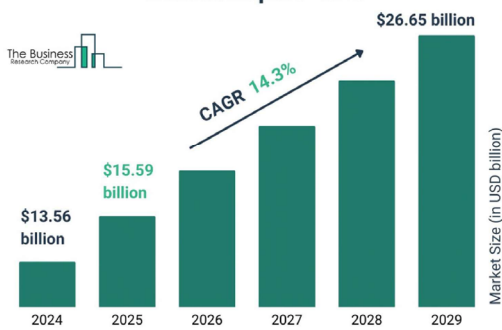
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植物育種和基因編輯植物的市場

Plant Breeding And CRISPR Plants Global Market Report 2025



The plant breeding and CRISPR plants market covered in this report is segmented –

- 1) By Type: Conventional breeding, Other Types
- 2) By Process: Selection, Hybridization and mutation breeding, Other Processes
- 3) By Application: Cereals and Grains, Oilseeds and Pulses, Fruits and Vegetables, Other Crops

Subsegments:

- 1) By Conventional Breeding: Selective Breeding, Hybridization, Mutation Breeding, Polyploidy Breeding, Other Types
- 2) By Other Types: CRISPR gene editing, Transgenic Breeding, Marker-Assisted Selection (MAS), Genome-Wide Association Studies (GWAS)

Plant Breeding And CRISPR Plants Market Size 2025 And Growth Rate

The plant breeding and CRISPR plants market size has grown rapidly in recent years. It will grow from \$13.56 billion in 2024 to \$15.59 billion in 2025 at a compound annual growth rate (CAGR) of 15.0%. The growth in the historic period can be attributed to increasing global population, crop disease outbreaks, food security concerns, regulatory changes, globalization of food trade.



Company Crops Innovation Newsroom Contact

Pioneering Non-Transgenic Trait Development

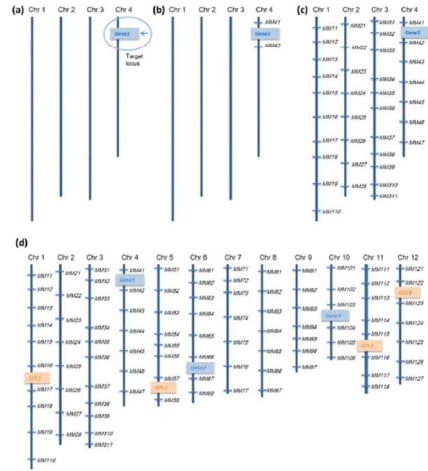
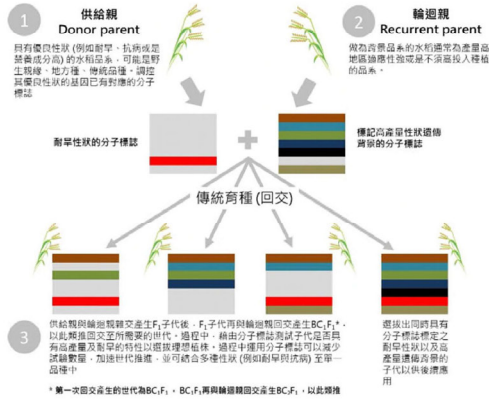
Our Rapid Trait Development System, *RTDS*SM, enables us to customize specific and predictable combinations of value-enhancing traits into plants within months rather than years. *RTDS* functions effectively as a "trait machine" enabling us to isolate a single plant cell, direct the desired edits without integrating foreign genetic material, and grow the entire plant from the single cell. We have developed the know-how to design traits that meet specific customer demands. In this regard, *RTDS* enables us to provide value to farmers and partners by combining multiple desirable traits ("stacked" traits) within the same plant. The following trait selection menu outlines the potential options for farmers and seed partners:

Parental or Elite Genetics	Herbicide Tolerant Traits	Other Grower Traits	Other Biotic Stress Tolerances	Abiotic Stress Tolerances	Consumer Traits
Choose Starting Germplasm / Variety <input type="checkbox"/> Elite Germplasm 1 <input type="checkbox"/> Elite Germplasm 2 <input type="checkbox"/> Elite Germplasm 3 <input type="checkbox"/> Other	Choose Herbicide Tolerance(s) <input type="checkbox"/> None <input type="checkbox"/> Mode of Action 1 <input type="checkbox"/> Mode of Action 2 <input type="checkbox"/> Other	Select all that apply <input type="checkbox"/> Pod Shelter Reduction <input type="checkbox"/> Seeding Maturity Early <input type="checkbox"/> Seeding Maturity Late <input type="checkbox"/> Other	Select all that apply <input type="checkbox"/> Pest Control 1 <input type="checkbox"/> Resistance to Disease 1 <input type="checkbox"/> Resistance to Disease 2 <input type="checkbox"/> Other	Select all that apply <input type="checkbox"/> Drought Resistance <input type="checkbox"/> Heat Tolerance <input type="checkbox"/> Other	Select all that apply <input type="checkbox"/> Healthier Oil <input type="checkbox"/> Allergen Free <input type="checkbox"/> Healthier Starches <input type="checkbox"/> Other

基因編輯種原 + 分子標誌輔助育種



分子標記輔助育種 Marker-Assisted Selection (MAS)



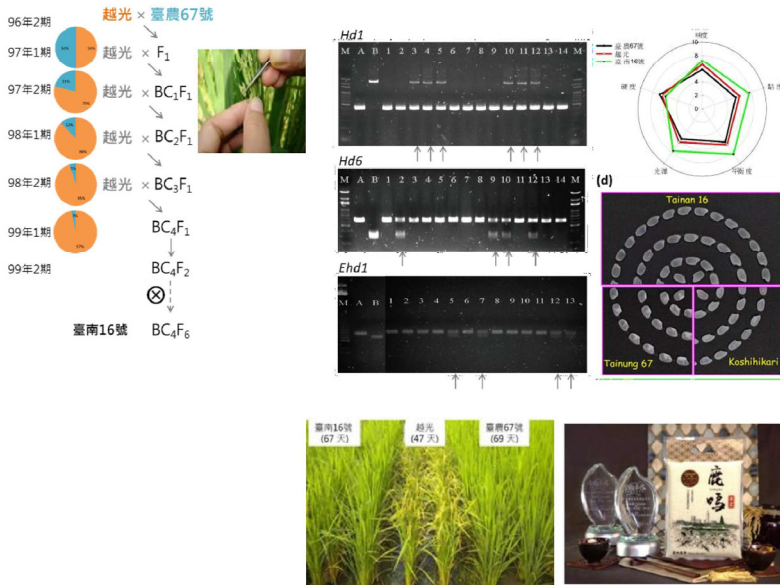
- > **前景選拔:** 針對標的基因做選拔
→ 提升標的基因的導入的育種效率
- > **背景選拔:** 依分佈在全基因體的分子標記做選拔
→ 提升恢復輪迴親 (優良親) 之基因的育種效率

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臺南16號

分子標記輔助回交育種 導入臺農67號抽穗期基因至越光



陳榮坤、林連芬、廖正宏(2012)水稻抽穗期基因Ta67-1的克隆。臺南區農業改良場研究報告60-1-12

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 GAP?

 基因在哪裡?



ChatGPT

drought resistance genes in rice

Key Drought-Resistance Genes in Rice

1. Transcription Factors (Regulators)

These are major controllers of stress-responsive pathways.

•DREB/CBF family

- DREB1/CBF, DREB2A – Activate drought-responsive genes via DRE/CRT elements; improve tolerance when overexpressed.

•NAC family

- SNAC1 (OsNAC9) – Enhances root architecture, stomatal control, and yield under drought.
- OsNAC6, OsNAC10 – Regulate stress signaling, strengthen roots, improve survival.

•bZIP family

- OsbZIP23, OsbZIP46, OsbZIP72 – ABA-signaling components improving drought resistance.

•AP2/ERF family

- OsERF48, OsERF71 – Regulate root growth and osmotic adjustment.

•MYB family

- OsMYB2, OsMYB4 – Improve osmotic stress tolerance and ABA sensitivity.

2. ABA Signaling and Osmotic Stress-Responsive Genes

•OsPYL/RCAR receptors (e.g., OsPYL5) – Gate ABA signaling, enhance drought responses.

•OsPP2C family – Negative regulators of ABA pathway; mutations can increase drought tolerance.

•OsDREB1F, OsLEA3-1 – Late embryogenesis abundant proteins that protect cellular structures.

資料探勘



資料探勘

3. Root Development and Architecture

Genes enhancing deep/robust root systems are central to drought avoidance.

- **DRO1 (DEEPER ROOTING 1)** – Controls root growth angle; introgressed into varieties like *IR64-DRO1* for improved drought yield.
- **WOX11/12** – Promote adventitious and crown root development.
- **OsNAC10 (root-specific expression)** – Increases root diameter and drought yield.

4. Water Transport (Aquaporins)

- **OsPIP1;1, OsPIP2;1, OsPIP2;2** – Plasma membrane intrinsic proteins regulating water uptake.
- **OsTIPs** (tonoplast aquaporins) – Maintain cellular water balance.

5. ROS Homeostasis and Stress Protection

- **OsAPX (ascorbate peroxidases), OsCAT (catalases)** – Detoxify reactive oxygen species.
- **OsEREBP1, OsSAP1** – Improve oxidative stress tolerance and survival.

6. Osmoprotectant Biosynthesis

- **OsTPS1, OsTPP** – Trehalose pathway genes stabilizing proteins and membranes.
- **OsP5CS1/2** – Proline synthesis genes enhancing osmotic adjustment.

7. Heat Shock Proteins (cross-tolerance with drought)

- **OsHSP70, OsHSP101, OsHSP17.0** – Protein folding and stabilization under dehydration/heat.

8. QTL-Associated Genes / Major Loci

Well-known drought-yield QTLs from upland or stress-tolerant rice:

- **qDTY2.1, qDTY3.1, qDTY12.1** – Large-effect QTLs enhancing yield under severe drought.
- Genes within these regions include regulators linked to root vigor, carbohydrate remobilization, and stress signaling.

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探勘資料

Gene / Locus	Main function / mechanism	Key experimental evidence	Breeding / biotech potential
DRO1 (DEEPER ROOTING 1)	Controls root growth angle → deeper roots for subsoil water capture	NILs with DRO1 show deeper roots and improved grain filling/yield under drought. Nature	High: used in marker-assisted introgression to improve drought avoidance and yield stability.
OsNAC10 (root-specific expression)	Promotes larger/thicker roots (stele, cortex) → increased water uptake	Root-specific overexpression increased root diameter and grain yield under field drought. OUP Academic	High: root-specific promoters useful to avoid growth penalties while improving yield.
SNAC1 / OsNAC (stress-responsive NAC)	Regulates stomatal closure, root development, and stress genes	Overexpression enhances drought/salt tolerance (reduced transpiration, better root traits). PNC	High: candidate for transgenic/edited lines and for pyramiding with root QTLs.
DREB2A (OsDREB2A)	AP2/ERF TF activating drought/osmotic stress genes (ABA-independent)	Overexpression improves drought tolerance in rice (physiological protection). Nature	Medium-High: strong stress response but needs careful expression control to avoid growth costs.
OsZIP23 / OsZIP46 (ABF/AREB family)	ABA signaling regulators; activate downstream drought/ABA genes	OsZIP23 overexpression increases drought tolerance and ABA sensitivity, OsZIP46 constitutive activation improves tolerance. PNC	High: modulate ABA responses; useful with tissue- or stress-inducible promoters.
OsPYLS (ABA receptor)	ABA receptor — modulates ABA signaling and stomatal/physiological responses	Overexpression of PYLS enhanced drought tolerance in rice. PNC	High: promising target for tuning ABA perception (genetic engineering or allele selection).
Aquaporins (OsPIP/OsTIP families)	Water transport across membranes → influences hydraulic conductivity	Multiple studies show PIP/TIP expression alters water flux and drought responses.	Medium: target for fine-tuning water use (careful tissue/context control needed).
ROS-scavenging enzymes (APX, CAT, SOD)	Protect cells from oxidative damage during dehydration	Overexpression/co-expression improves stress tolerance in transgenic plants.	Medium: good as part of multi-gene stacks to reduce damage.
Osmoprotectant biosynthesis (P5CS, TPS/TPP)	Accumulation of proline/trehalose for osmotic adjustment	Transgenic lines with elevated proline/trehalose show better dehydration tolerance.	Medium: metabolic tradeoffs must be managed.
qDTY family (qDTY2.1, qDTY3.1, qDTY12.1, etc.)	QTLs that consistently increase grain yield under drought	qDTY loci repeatedly detected and used in marker-assisted breeding for drought yield. PNC	Very high: directly used in MAS to improve drought yield performance.

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探勘資料

Key CRISPR/Cas Drought-Editing Cases in Rice

1. OsERA1

- Using CRISPR/Cas9, rice plants with frameshift mutations in OsERA1 showed **enhanced response to ABA (abscisic acid) and improved drought tolerance**, with better stomatal regulation and primary root growth. [PLOS](#)
- Implications: OsERA1 is a negative regulator of drought/ABA responses → editing it reduces that repression, improving tolerance without a major penalty on leaf growth.
- Breeding/biotech note: Good target for editing to boost root + ABA responsiveness, but need to check field/yield trade-offs.

2. OsDST (Drought and Salt Tolerance gene)

- CRISPR/Cas9 was used in an indica cultivar (MTU1010) to generate a ~366 bp deletion in OsDST. The mutant had broader leaves, reduced stomatal density, better water retention under dehydration. [PubMed](#)
- Mechanism: By reducing stomatal density (and presumably reducing transpirational water loss) the edited lines show improved tolerance.
- Note: The study focuses on seedling/controlled stage; for breeding you'll need performance data under field drought.

3. OsPYL9

- Using CRISPR/Cas9 to knock out or modify OsPYL9 (an ABA receptor) led to higher cuticular wax accumulation, lower transpiration rate and improved grain weight under drought. [PMC+1](#)
- Mechanism: Modulating ABA signalling via PYL receptor influences physiological drought responses (wax, stomata/transpiration).
- Implication: A very promising target for combining drought avoidance (water conservation) and yield retention.

4. Other candidate genes / negative regulators

- A recent review highlights many other genes edited by CRISPR for drought tolerance in rice: e.g., OsWRKY5 (knock-out increases drought tolerance) and others like OsSRL1, OsSRL2. [Frontiers+1](#)
- Broadly: many of these are *loss-of-function* edits of negative regulators of drought/adaptation, which is a recurring theme.
- Also, a meta-review reports CRISPR-based editing of genes for root development, stomatal density, osmotic/oxidative stress responses in rice. [PMC](#)

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探勘資料

Practical Considerations for Your Research/Breeding

- **Target gene selection:** Focus on genes with proven functional roles in drought/ABA responses (e.g., OsERA1, OsDST, OsPYL9).
- **Phenotyping under field-like drought:** Editing shows promise in seedlings/controlled conditions but verifying under realistic drought + yield conditions is essential (especially given your interest in grain quality, heat tolerance etc.).
- **Trade-offs:** Some edits (e.g., stomatal density reduction) may reduce photosynthetic capacity, yield or adaptation to other stresses—so multi-trait tests are needed. For example, one study edited the stomatal regulator EPFL10 to reduce stomata gently (rather than radically) for improved water-use efficiency. [Innovative Genomics Institute \(IGI\)+1](#)
- **Stacking strategies:** Given your focus on multiple crops and traits (flowering time, grain quality, heat tolerance), combining CRISPR targets (drought + heat + quality) may be more future-proof.
- **Regulatory & breeding pipeline:** Ensure created edits are free of off-target effects, segregate out Cas9 if necessary (for regulatory/acceptance issues) and integrate edited alleles into elite varieties (for example, via backcrossing or direct editing in elite background).
- **Molecular & physiological read-outs:** As you serve as editor for plant science journals, ensure your experiments report detailed ABA signalling assays, stomatal conductance, root architecture, yield components, and ideally multi-season/field data.

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探勘資料

Plant Mol Biol Plant (June 2020) 20(6):1099–1110
https://doi.org/10.1007/s12298-020-00819-w

RESEARCH ARTICLE

CRISPR-Cas9 mediated genome editing of drought and salt tolerance (*OsDST*) gene in *indica* mega rice cultivar MTU1010

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資料探勘

如何使用 CRISPR-Cas9 增強水稻的耐旱和耐鹽性?

利用 CRISPR-Cas9 技術增強水稻的耐旱性和耐鹽性，主要是透過精準編輯與非生物逆境耐受性相關的基因，從而產生具有所需表型特徵的突變體。根據所提供的研究，CRISPR-Cas9 被用於編輯水稻中的抗旱和耐鹽性基因 (*OsDST*)，目的是在主要種植的籼稻品種中創造出具有理想等位基因的精英品種。以下是使用 CRISPR-Cas9 增強水稻耐旱性的具體步驟和機制：

1. 靶向與基因編輯 (Gene Editing)
 - 選擇目標基因： 研究人員選擇了 *OsDST* (Drought and Salt Tolerance, 抗旱和耐鹽性) 基因作為候選基因。*OsDST* 基因編碼一個鋅指轉錄因子。
 - CRISPR-Cas9 系統的應用： 該研究使用了基於非同源末端接合 (NHEJ) 突變方法的 CRISPR-Cas9 系統進行基因編輯。NHEJ 是一種易出錯的修復機制，會導致雙股 DNA 斷裂處發生缺失或插入突變。
 - 設計與轉化： 設計了兩個不同的導引 RNA (gRNA)，旨在靶向 *OsDST* 蛋白中可能涉及蛋白質-蛋白質相互作用的區域。隨後，將包含 gRNA 和 Cas9 表達盒的重组二元載體 (例如 pC1300-PosU3::sgRNA1-PUG::SpCas9n-PosU3::sgRNA2) 引入到農桿菌 (*Agrobacterium tumefaciens*) EHA105 菌株中，用於轉化籼稻品種 MTU1010 的癒傷組織。
 - 產生特定缺失突變體： 成功產生了 *DST* 基因的不同突變等位基因。研究人員選擇了一個純合突變體，該突變體在兩個 gRNA 的 PAM 位點之間發生了 366 bp 的缺失。這個突變體命名為 *dstD184-305*。
2. 突變帶來的耐受性機制

dstD184-305 突變是一種功能喪失型突變 (loss-of-function mutant)，它模擬了先前在日本型水稻品種中報告的由 EMS 誘導的 *dst* (N69D) 突變的表型。耐旱性增強的機制主要包括：

 - A. 氣孔密度減少與保水性增強
 - 氣孔密度下降： *dstD184-305* 突變體相較於野生型 MTU1010，氣孔密度顯著降低。
 - 基因下調： 氣孔密度的減少，至少部分歸因於氣孔發育基因 *SPCH1*、*MUTE* 和 *ICE1* 的表達量下調。這表明 *DST* 蛋白可能直接或間接正向調控這些氣孔發育基因。
 - 保水性提高： 由於氣孔密度減少，在脫水脅迫下，*dstD184-305* 突變體的葉片展現出增強的葉片保水能力。在離體葉片失水試驗中，突變體的失水率顯著降低。
 - B. 逆境耐受性表現
 - 耐鹽性高： 在幼苗階段的逆境耐受性試驗中，*dstD184-305* 突變體對 200 mM NaCl 鹽脅迫表現出高度耐受性。
 - 經歷鹽脅迫恢復期後，突變體幼苗的存活率超過 65%，而野生型 (WT) 僅存活約 20%。
 - 在鹽脅迫下，突變體的葉綠素保留量也顯著更高。
 - 耐滲透壓脅迫 (類乾旱)： 該突變體對 20% PEG6000 誘導的滲透壓脅迫表現出中等程度的耐受性。在脅迫恢復後，突變體的幼苗生物量顯著高於野生型。

總括簡而言之，使用 CRISPR-Cas9 增強水稻耐旱和耐鹽性的過程，是通過精確創製 *DST* 基因的功能喪失型突變體 (*dstD184-305*)。這種突變體通過下調氣孔發育基因，大幅減少葉片上的氣孔密度，從而減少水分散失並增強耐旱性，同時也顯著提高了對鹽脅迫的耐受性。透過 CRISPR-Cas9 編輯水稻的 *DST* 基因，就像是給水稻安裝了一個「節水閥門」：當這個基因被關閉時 (功能喪失突變)，它會使得葉片表面的氣孔數量減少，從而限制了水分蒸發，讓水稻在乾旱和高鹽等缺水環境中更能「守住」自己寶貴的水分。

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探勘新基因

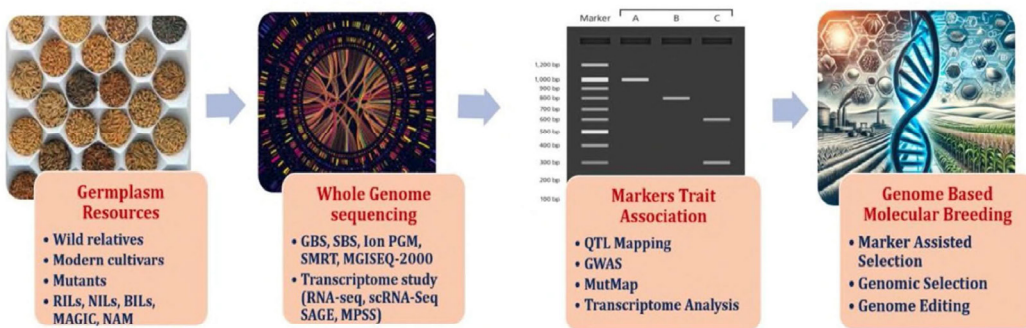
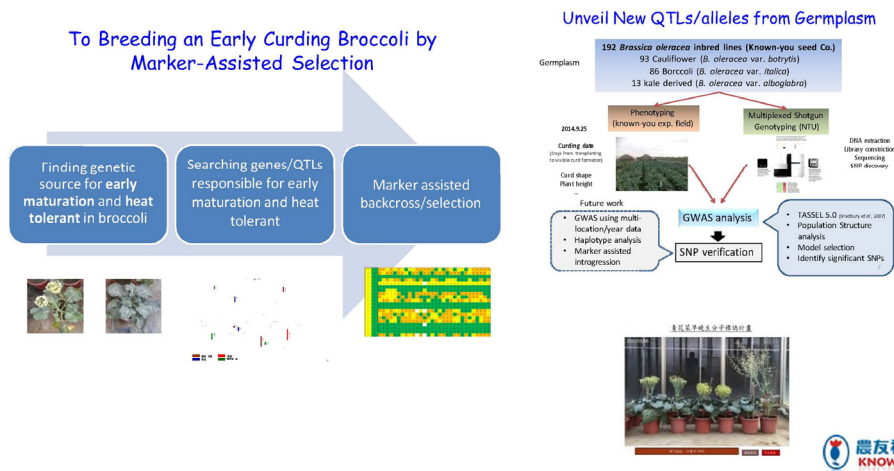


Fig. 2 Trait discovery and genome based molecular breeding

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探勘甘藍類作物始球期基因





探勘甘藍類作物始球期基因

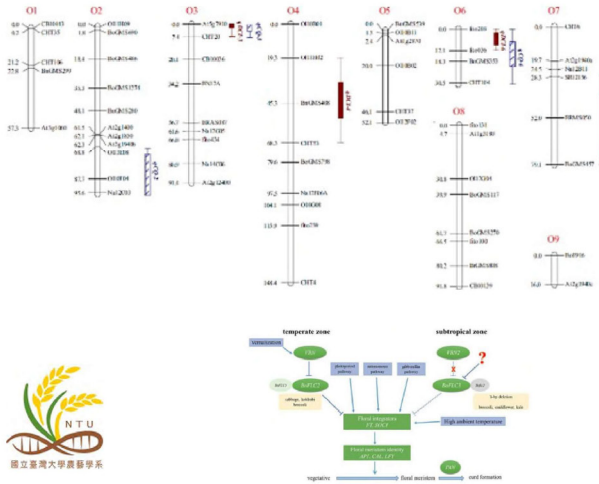
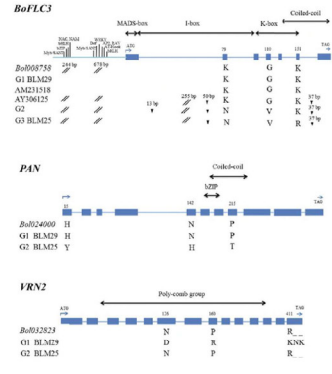


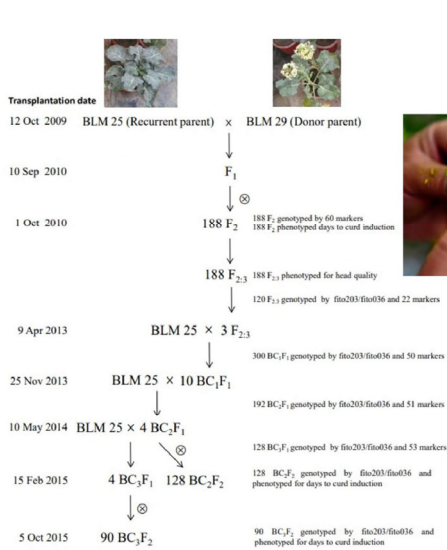
Figure 4. A model for genetic and environmental control of flowering time and curd formation in temperate versus tropical *B. oleracea*. The crops, flowering pathways, and allelic genes are indicated by yellow, blue, and green, respectively.



Lin et al. (2018)



花菜始球期之分子標誌輔助選育



Supplementary Figure 2 QTL mapping of days to curd induction (DCI) and curd quality (CQ)

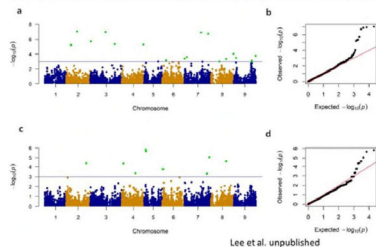




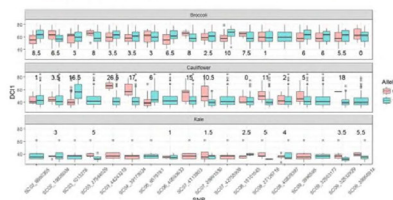
探勘甘藍類作物始球期基因

全基因體關連性分析

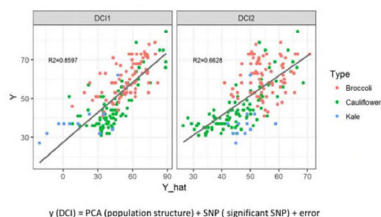
Genome-Wide Association Studies



The Allelic Effects of the Significant SNPs Associated with DCI



The Association of the Significant SNPs with DCI



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GAP?

性狀 (V_p) = 基因 (V_g) + 環境 (V_e)

遺傳力 (h^2) = 基因 (V_g) / 性狀 (V_p)

✿ 耐熱、耐旱與耐澇

✿ 成熟期、株高

✿ 產量

✿ 品質

✿ 營養

$$V_g = V_a + V_d + V_{g \times g} + V_{g \times e} + V_{g \times m}$$

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數位育種

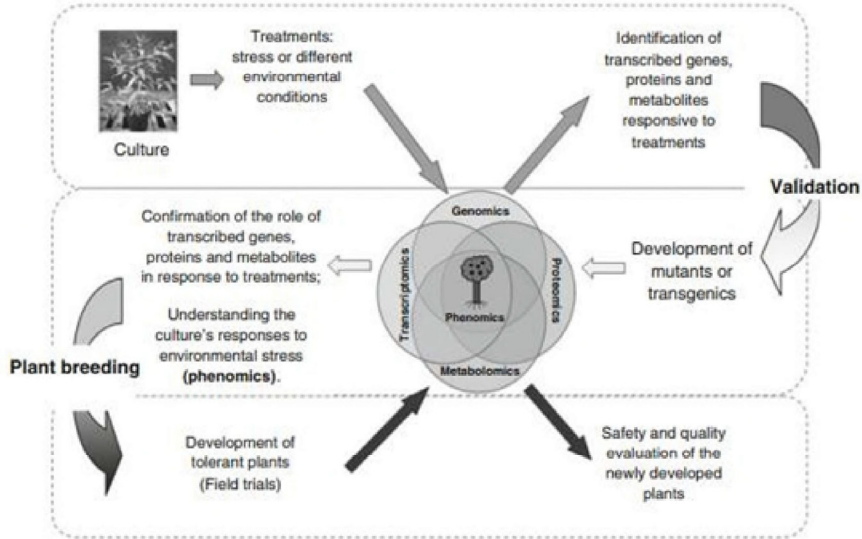


Fig. 8.3 Application of “omics” in plant breeding programs: identification of genes, proteins, and metabolites, and their validation relative to function and applicability. Adapted from Abreu et al. (2013)

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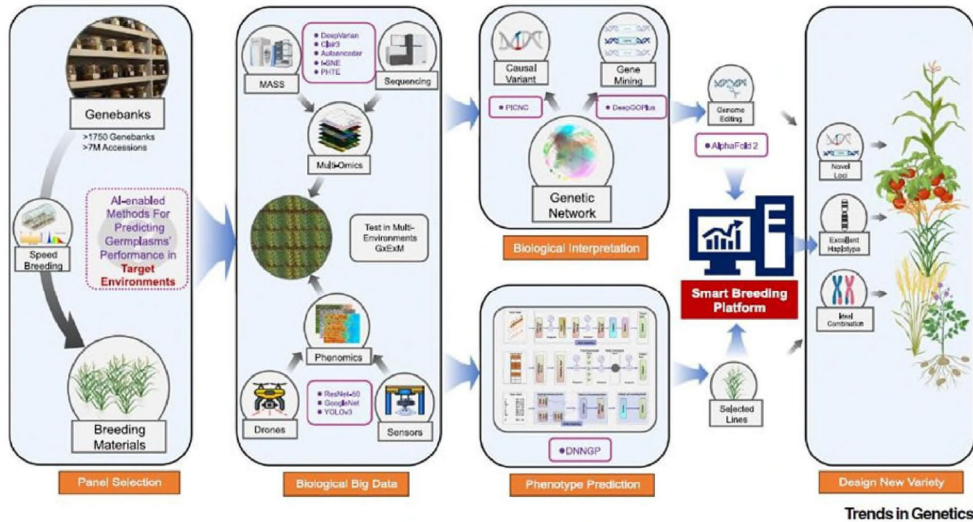


Figure 1. A roadmap of artificial intelligence (AI)-enabled plant breeding. The far left panel depicts the source of germplasm from a genebank that is either used directly or subjected to breeding to utilize omic analyses to generate big data (second panel). The big data from the germplasm could uncover genes and predict breeding values (third panel), which would characterize the gene-editing targets and optimal lines for developing next-generation cultivars. Representative AI-based methods are identified in purple boxes. The dotted box represents methods that require development.

Farooq et al. (2024) Trends in Genetics 40: 891

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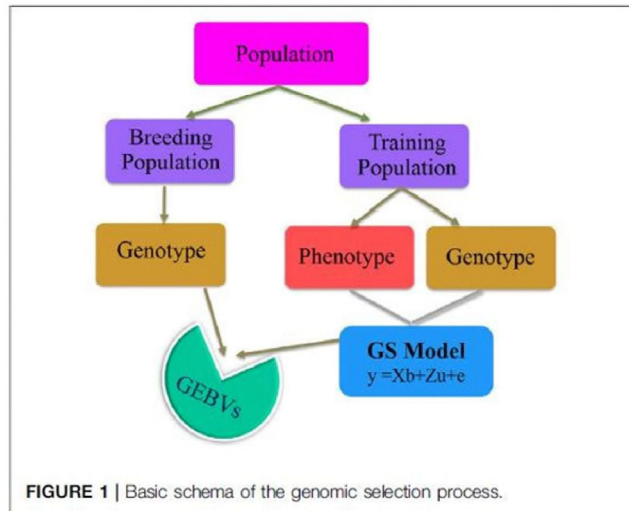
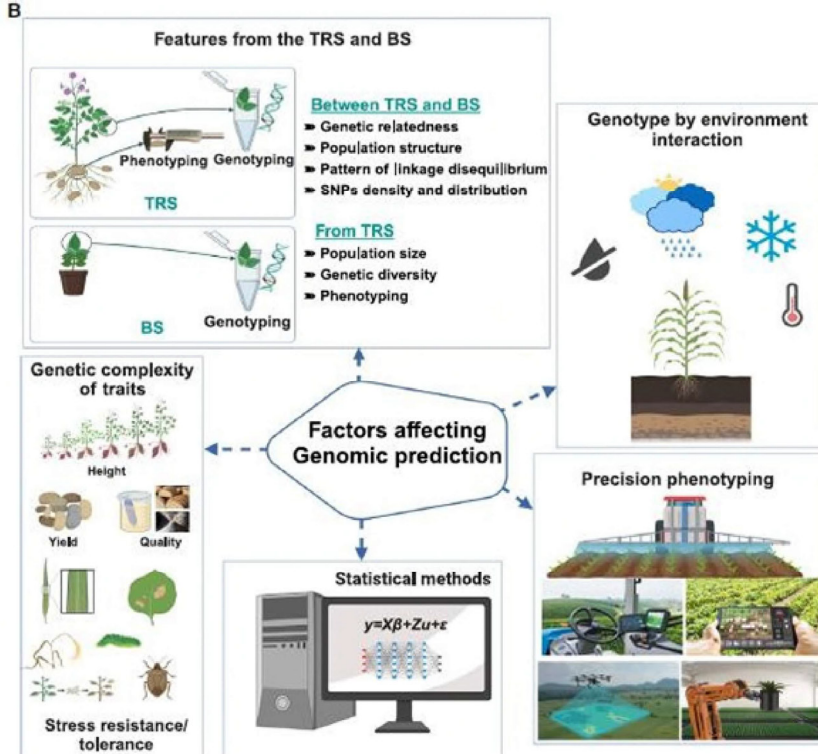
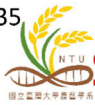


FIGURE 1 | Basic schema of the genomic selection process.

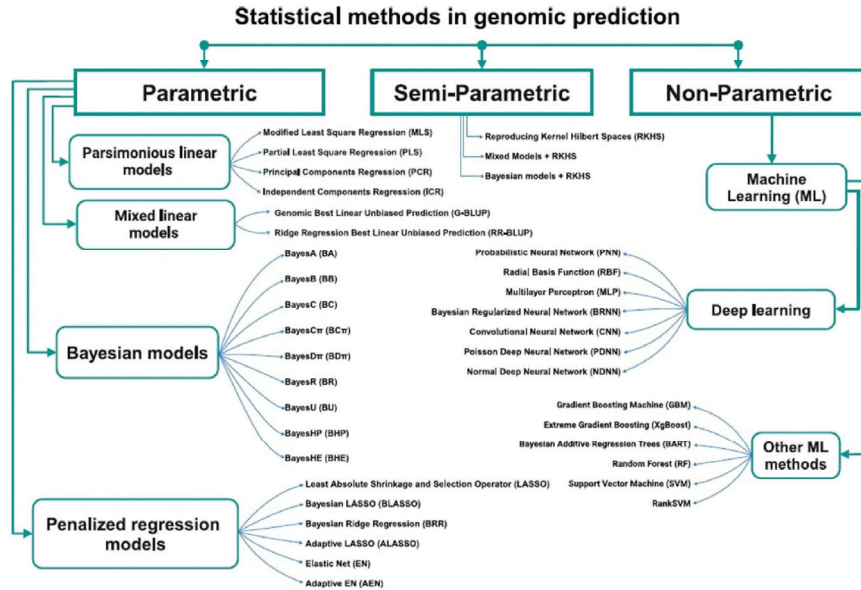
In this approach, the individual effect of each marker is estimated, and the additive sum of all the marker effects is used for calculation of the **genomic-estimated breeding values (GEBV)** of each individual. In the current scenario of climate change, GS is a promising tool for improving the genetic gain of individuals under the breeding program (Yuan et al., 2019). The basic process of any genomic selection process starts with the creation of training population, i.e., individuals having both genotypic and phenotypic information, and this information is used to build a model, where the phenotype is used as a response and genotype as a predictor. The information from the developed model is later used to estimate the GEBV of breeding population, i.e., individuals having only genotypic information.



Alemu et al. (2024) Molecular Plant 17, 552–578



Statistical Methods in Genomic Prediction



《因應氣候變遷之水稻與雜糧未來研究論壇》臺中區農業改良場 2025年11月26日 Alemu et al. (2024) Molecular Plant 17, 552–578



Genomic Selection

- ✿ Genomic selection, the application of genomic prediction (GP) models to select candidate individuals, has significantly advanced in the past two decades, effectively accelerating genetic gains in plant breeding.
- ✿ We delved into the pivotal roles of training population size and genetic diversity, and their relationship with the breeding population, in determining GP accuracy.
- ✿ The density and distribution of single-nucleotide polymorphisms, level of linkage disequilibrium, genetic complexity, trait heritability, statistical machine-learning methods, and non-additive effects are the other vital factors.
- ✿ The search for high accuracy in GP—theoretically reaching one when using the Pearson’s correlation as a metric—is an active research area as yet far from optimal for various traits.
- ✿ We hypothesize that with ultra-high sizes of genotypic and phenotypic datasets, effective training population optimization methods and support from other omics approaches (transcriptomics, metabolomics and proteomics) coupled with deep-learning algorithms could overcome the boundaries of current limitations to achieve the highest possible prediction accuracy, making genomic selection an effective tool in plant breeding.



育種之新趨勢

✿ 跨領域人才

- Genome Designer
- 農園藝學
- 植物科學
- 生物資訊
- 資料科學