



新興花卉品種之育種策略及技術應用

國立中興大學園藝學系

溫佩容、李若瑋、陳彥銘

■ 摘要

本文探討了新興花卉品種育種策略，並聚焦於遠緣雜交、誘變育種、原生質體技術、基因轉殖和基因編輯等育種技術應用。透過遠緣雜交技術，可跨越物種界限，創造具有獨特適應性和生物多樣性的新品種。誘變育種則利用輻射或化學方法引發遺傳變異，快速擴充植物性狀變異。原生質體技術進一步突破生殖障礙，開發全新花卉品種。基因轉殖與基因編輯技術則可輔助育種家精確調控植物基因，優化花卉性狀如花色、香味及抗病力。本文將闡述傳統和現代育種技術的發展及沿革，展示近年來新興花卉育種成果、以及說明這些育種技術及成果如何推動花卉產業的創新發展。

關鍵詞：遠緣雜交、誘變育種、原生質體、基因轉殖、基因編輯

■ 前言

在全球花卉市場持續增長的驅動下，創新的育種技術對於開發新品種具有至關重要的作用。本文聚焦於新興的育種策略和技術，包括遠緣雜交、誘變育種、原生質體技術、基因轉殖和基因編輯等方法。這些技術使得育種家能夠克服自然界的限制，例如物種間的生殖障礙，並快速開發具有商業和美學價值的新型花卉品種。遠緣雜交結合遠距離物種的遺傳物質，促進基因的新組合和多樣性。誘變育種則透過人為誘導遺傳變異，快速生成多樣的性狀變異。原生質體技術的應用突破了傳統的物種界限，進一步豐富了育種材料的來源。而基因轉殖和基因編輯技術的發展，為精確調控植物性狀提供了可能，從根本上增強了育種的效率和準確性。這些先進技術的集成不僅推動了花卉特性的快速改良，也為花卉育種帶來了革命性的進步。

■ 利用遠緣雜交創造新興花卉品種

雜交是所有生物（如動物、植物、真菌）進化重要的程序步驟之一（Gross and Rieseberg, 2005、Mallet, 2007、Schwenk *et al.*, 2008、Mavarez and Linares, 2008、Giraud *et al.*, 2008、Paun *et al.*, 2009、Soltis and Soltis, 2009）。已有諸多文獻支持，透過雜交可能創造同倍性（Rieseberg *et al.*, 2003、Gompert *et al.*, 2006）或非預期基因組組合（Cronn and Wendel, 2004、Chen and Mii, 2012）的案例，因此在雜交過程中，多倍體對植物演化或花卉育種的影響，應該一併考量。相較於突變方式，透過雜交育種方式對於遺傳物質的改變較為顯著，更具效率（Stebbins, 1959、Knobloch, 1972），並能增加物種或品種的適應力（Whitney *et al.*, 2006、2010、Campbell *et al.*, 2009），甚至突破基因屏障，對於外表型改變產生關鍵性的影響（Kalisz and Kramer, 2008）。然而透過品種間雜交，其有限的基因背景已無法滿足育種者的需求。此外因品種選拔主要透過符合育種目的進行人為挑選，極易在子代中忽略及丟失可能具有的潛力遺傳物質（如耐熱、抗病）。因此透過遠緣雜交重新導入血緣，抑或是輸入新穎種質資源，均可對該物種 / 品種產生突破性的影響。

遠緣雜交後代通常能夠表現比親本生長速度更快，生物量更大、甚至繁殖力更強的植物特徵，早期已被歸因可能與雜種優勢相關（Kölreuter, 1766）。此外在雜交演化過程中，異交植物為避免同源性隱性等位基因組合，容易造成致病 / 致命的遺傳物質，對於外來花粉接納性較大，因而可創造多樣性異質性基因組合。近年來透過遺傳學和基因組學方法，已證實造成多樣性基因組異質性的機制，可能與等位基因之間的相互作用、基因組的遺傳修飾、以及 small RNAs 的活性相關（Chen, 2013），此外透過數量性狀基因座（QTL）也可被用來識別導致異質性表型的基因座，然後確定其特徵，對於加速育種程序，提升育種效率，已在商業界廣泛利用及運行（Tang *et al.*, 2010、Zhou *et al.*, 2012、Shen *et al.*, 2014、Shang *et al.*, 2015）。



然而雜交過程可能因生殖障礙（合子前 & 合子後）的產生，導致雜交後代敗育或無法產生，因此透過相關技術解除生殖障礙已成為創造新穎雜交後代至關重要之關鍵因素。目前透過解剖學上觀察（Hawkins *et al.*, 2016），可詳細得知花粉受精後精核抵達胚珠過程中是否產生障礙，抑或是雙重受精後，合子發育是否有產生敗育情況，以上均可有對應性的育種技術及策略進行解除（邱和王，2011）。透過上述前趨的釐清與瞭解，更能夠有效創造雜交後代。

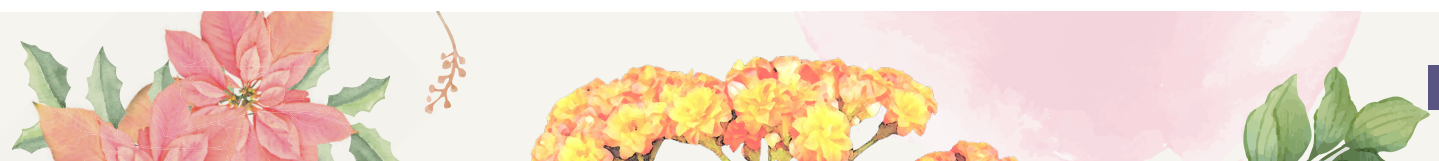
近年來透過遠緣雜交創造新形態花卉新品種，已扮演重要的角色，如蝴蝶蘭與狐狸尾蘭之屬間雜交後代（蔡和翁，2014、Jitsopakul *et al.*, 2022）、矮牽牛與舞春花之屬間雜交後代（葉，2021）、火焰百合與宮燈百合之屬間雜交後代（Amano *et al.*, 2009）、石竹屬與滿天星之屬間雜交後代（Nakano *et al.*, 1996）、以及多種花卉作物利用種間雜交方式獲得新品種的成功案例，提供花卉市場突破性植物性狀，並為未來花卉育種策略，創造新的種質資源、為育種藍圖增添一抹新的色彩。

■ 新興誘變育種技術對觀賞作物育種之影響

誘變育種方式為生物遺傳學重要研究及突破之一，Stadler（1928a, b）首次證明輻射可用於誘導及增加植物遺傳變異機率。但實際應用則始於 Friesleben and Lein（1942）發表文章後，才有大規模利用誘變技術進行植物育種。Gustafsson（1947a, b）、Hoffmann（1959）和 Mackey（1956）進行了系統的研究，並獲得了有關最佳放射線誘變之處理劑量、處理條件、突變頻率和突變譜的資訊。Nilan *et al.*（1965）則分析及統整突變的輻射處理條件和綜合處理方法。自 1960 年起，誘變育種已被廣泛利用於作物改良。誘變育種且被認為是基於一個完成產品（如品種），透過增加突變機率修飾及改變植物性狀，也是誘導優秀栽培品種產生衍生品種發生的途徑之一。

誘變育種有許多方式，其中物理誘變包含 X 射線、伽馬射線（急性和慢性）、中子（快和熱）、電子、光子、 α 射線以及 β 射線。而許多化學藥品也兼具誘變力，如烷化劑、抗生素等，常用的化學誘變劑有甲烷磺酸乙酯（EMS）、甲烷磺酸甲酯（MMS）、硫酸二乙酯（dES）、乙烯亞胺（EI）、亞硝基脲乙酯（ENU）、亞硝基脲甲酯（ENH）、亞硝基脲甲酯（MNH）、疊氮化物等。由於各化學誘變劑其突變率與作物種類存在依賴性，且實際應用上存在許多問題（如處理方式、供體種類、安全性、重現性差、藥劑誘變之持久性）。因此，化學誘變劑之應用逐漸式微，而物理誘變之研究及應用仍持續進行。

誘變技術創造及開發花卉新興品種已成功應用於孤挺花、百合、九重葛、美人蕉、菊花、大理花、非洲菊、唐菖蒲、玫瑰、晚香玉、水仙等花卉作物，並創造不同花色及植物性狀變異（Datta, 1988、1989、1992、1994、1997、2000、2004、2005a、b、2009a、b、c、2015a、b）。例如透過伽馬射線可創造玫瑰花不同型態特徵如花色變異



之突變品種 (Pandey and Datta, 1995)。經統計，誘變育種已被利用於 170 多種不同植物物種，並創造 3218 個突變品種 (Datta, 2023)。其主要原理就是利用物理和化學誘變劑可誘導細胞和組織發生細胞學、形態學、生理學和遺傳學變化 (Beal and Scully, 1950、Bowen and Cawse, 1962、Chadwick and Leenbouts, 1981、Davis and Wall, 1961、Etter, 1965、Evans, 1962、1966、Ford, 1948、Gunckel, 1957、Kihlman, 1966)，進而產生性狀變化。

此外新興誘變技術也不斷研發。Ion beam (重離子) 為近年被廣泛應用於觀賞作物的誘變技術。目前可利用的重離子種類有：雷射光束照射 (氦氖鐳射, He-Ne)、氦 (He)、碳 (C)、氖 (Ne) 和氬 (Ar)，其中又以 220 MeV 的碳離子最具效率且突變頻率較高，可創造多樣的花色、花型、及新穎的突變性狀。如菊花、康乃馨、仙客來、大理花、飛燕草、矮牽牛、薔薇均已被證實 (Abe *et al.*, 2018、Azad *et al.*, 2018 a, b、Li *et al.* 2018a, b)。此外為了克服傳統誘變方式其干擾染色體之隨機方式，並配合現今科學研究對基因調控性狀之背景瞭解，進而產生精準型之誘變育種方式：『定向誘導基因組局部突變技術 (Targeting Induced Local Lesions In Genomes, TILLING)』。且由於生物基因組定序成本降低，此方法可快速鑒定基因突變的植物 (Alonso and Ecker, 2006、Ostergaard and Yanofsky, 2004、Wang *et al.*, 2009a, b)。TILLING 技術結合傳統誘變技術和基因背景鑑定技術，可以提升篩選具優良改良性狀之突變體效率 (Amri-Tiliouine *et al.*, 2018、Colbert *et al.*, 2001、Eliot *et al.*, 2008、Laouar *et al.*, 2018)。此外透過『內部標記進行核酸內切突變分析 (Endonucleolytic Mutation Analysis by Internal Labeling, EMAIL)』，可提升檢測樣本其突變基因的靈敏度。相較於 TILLING 方法，EMAIL 技術提供育種者，可於植物進行田間試驗前進行早期篩選，並針對特定基因的誘導突變所產生之變異體進行選拔 (Caldwell *et al.*, 2004、Comai and Henikoff, 2006、Comai *et al.*, 2004、Cordeiro *et al.*, 2006、Cross *et al.*, 2008、Gilchrist *et al.*, 2006a, b、Henikoff and Comai, 2003、Lee *et al.*, 2009a, b、Mejlhede *et al.*, 2006、Nieto *et al.*, 2007、Oleykowsky *et al.*, 1998、Sato *et al.*, 2006、Slade and Knauf, 2005、Till *et al.*, 2010)。

■ 利用原生質體技術創造花卉新品種

由於花卉品種之求新求變特性，傳統採以現有種質資源進行植物特性遺傳之方式，已無法滿足育種者之想像。然而生殖障礙等諸多因素，造成物種間無法順利產生後代，因此新型育種技術之引入變成是一件必要且重要的策略及手段。透過原生質融合技術，可突破物種間隔離障礙。此外藉由原生質融合技術生殖行為，可透過對稱融合或非對稱融合方式，藉以瞭解其遺傳行為，並獲得傳統育種無法獲得之雙細胞質融合體，進而創造新穎種質資源以供後續育種材料使用。

然而使用原生質融合技術，其前提必須建立完善之細胞分離、細胞培養、細胞融



合等原生質培養系統。利用『細胞全能分化性』理論，單一細胞有其能力發展成完整植物個體。然而許多因素仍會影響使用原生質分離、培養及融合效率，目前已有文獻證實，從細胞分離至芽體再生過程存在諸多的障礙（Andersson *et al.*, 2018、Murovec *et al.*, 2018、Svitashev *et al.*, 2016、Xia *et al.*, 2020、Yu *et al.*, 2021）。此外，原生質體分離、培養及再生也被證實存在物種及品種依賴性（Adedeji *et al.*, 2020、Cui *et al.*, 2019、Kang *et al.*, 2020、Meyer *et al.*, 2009、Nassour and Dorion, 2002、Tomiczak *et al.*, 2016），並非單一標準流程就可套用於所有後續相關試驗，必須針對優化等措施。因此原生質技術發展相較於其他育種技術，顯的困難重重且緩慢。

雖然進度緩慢，但原生質融合技術等相關研究依舊持續進行。目前已有玫瑰花、菊花、香石竹、矮牽牛、龍膽、繡球花、百合進行相關研究（Afkhami-Sarvestani *et al.*, 2012、Furuta *et al.*, 2004、Horita *et al.*, 2003、Kästner *et al.*, 2017、Nakano and Mii, 1993、Pati *et al.*, 2008、Power *et al.*, 1980、Tomiczak, 2020、Tomiczak *et al.*, 2017）此外透過原生質融合方式，可發現一些新穎植物特性及現象，如菊花之抗病及花型花色改變（Furuta *et al.*, 2004），以及石竹與滿天星異屬之融合體（Nakano *et al.*, 1996），其子代植株矮化且連續開花等特性。

此外藉由原生質體可透過物理及化學方式直接吸收 DNA、RNA 或蛋白質的特性（Duarte *et al.*, 2016、Shen *et al.*, 2017、Xu *et al.*, 2020），因此結合基因編輯技術，為近年來利用原生質體方式之新興育種技術及方式。透過此方式，也已被證實可在數小時至數日間獲知基因編輯效率（Subburaj *et al.*, 2016、Xu *et al.*, 2020、Xia *et al.*, 2020、Yu *et al.*, 2021），顯示原生質體技術除了進行融合外，搭配不同育種技術，亦能協助育種成果發展。

因此利用原生質體技術，除可突破生殖障礙創造新穎體細胞雜交體外，亦可加速基因編輯技術，對於觀賞花卉育種及改良遺傳，為一種有用的工具及策略。然而利用此技術前，必須建立及完善所有操作步驟，甚至針對單一試驗進行調整及優化，此為關鍵因素。原生質體技術已發展多年，發展相較於其他育種技術雖無一日千里之效，但隨著其他育種技術的不斷發展，原生質體技術依舊扮演其關鍵角色。顯見育種技術並無新舊之分，善用其原理及方法，與時俱進，便可創造及提升 1+1>2 之育種功效（Naing *et al.*, 2021）。

■ 基因轉殖在花卉作物上之應用

基因轉殖技術已被廣泛利用於多種大量生產之農園藝作物，自 1983 年後農桿菌腫瘤誘導（Ti）轉殖技術發表後（O'Brien, 1983），轉基因作物已可於 41 個國家種植（English and Schreiber, 2020），顯見其對產業之重要性。花卉及觀賞作物於園藝產業扮演重要角色，其多樣性之產品型態（切花、盆花、種苗、種子、球根等），並透



過後續應用（花藝、景觀）衍生其經濟價值。然而物種基因限制，部分預期性狀無法透過傳統育種或雜交方式育成，因此透過基因轉殖導入外源基因或進行性狀修飾，已被廣泛利用於花卉作物。其應用範圍可能改良花的形態、新的花色、誘導早花、增強香味或延長壽命、抗逆性或抗病性等。而應用的植物種類範圍，經 Boutigny *et al.* (2020) 文獻統計，目前已超過 50 種以上的花卉作物已被進行相關研究，其中蘭科植物就佔了 113 篇發表文獻。而花卉又以菊花 (26.7%)、矮牽牛 (15.2%)、蘭科植物 (6.7%)、玫瑰 (6.7%)、石竹 (5.5%) 和香椿 (5.5%) 為大量研究文獻發表的作物種類。而在這些發表文獻中，對花色的改良研究最多，占 29.1%。其他重要的轉基因性狀包括形態 (12.7%)、壽命 (12.1%)、早花 (8.5%)、抗真菌和病毒 (7.9%)。此外 10.3% 的研究發表也指出透過基因轉殖可增加觀賞植物的性狀多樣性。因此以上證據顯示，透過基因轉殖，不但可有利於我們對於基因背景之調控及理解，更能加速拓展花卉產品之未來可能性。

此外基因轉殖對於花卉作物之相關研究，並非僅是紙上談兵，近年來透過基因轉殖，釋放至花卉市場之新興花卉品種也陸續增加，從最早期的藍色玫瑰花 (Suntory Flowers 公司 Applause®) (Kishi-Kaboshi *et al.*, 2018)、藍色康乃馨 (Florigene 公司 Moonseries®) (Tanaka and Brugliera, 2013)、藍色菊花 (Suntory Flowers 公司 Blue Ocean®) (Noda, 2018)、藍色蝴蝶蘭 (Wedding Promenade 'Blue Gene'®) (Mishiba *et al.*, 2005)、螢光矮牽牛 (Light bio®)(Bourzac, 2024)、螢光蝴蝶蘭 (Mii and Chin, 2024) 也一一於相關市場銷售。由於其植物特徵新奇，對市場吸引力強烈，銷售容易，且經多年市場流通後，消費民眾對於此基因轉殖之花卉作物之接受度也越來越高，因此顯見基因轉殖技術對於花卉作物之應用性應大有作為。

新興之基因轉殖技術，近年來也不斷進行研究，藉以提昇轉殖效率。早期基因轉殖方式，可透過間接基因轉化和直接基因轉化 (Keshavareddy *et al.*, 2018)。間接遺傳轉化是利用生物作為載體的一種方法，如農桿菌介導的基因轉移到靶細胞中；而直接遺傳轉化則是利用外力將目的基因傳遞到植物細胞中，包括粒子轟擊/基因槍、電穿孔、脂質體、碳化矽、顯微注射和花粉管途徑介導的植物遺傳轉化方法 (Klein, 2011; Rao *et al.*, 2009)。然而以上方式，均需透過良好之組織培養技術方能達成。因此簡便之新興轉殖技術也於近年發表，可突破受物種限制，僅需利用植物營養器官於外界再生之概念進行轉殖即可獲得大量且穩定之轉殖子代 (Cao *et al.*, 2022)。因此未來透過此方式，應可加速基因轉殖於花卉作物之應用。

■ 基因編輯技術利用於觀賞作物的應用

育種技術的不斷進化，開發高效率之育種方式已是需求。儘管傳統雜交育種方式如種內雜交、種間雜交、誘變育種、多倍體化、雙單倍體培養，已能為我們帶來許多



新穎特性及品種，然而一些顯而易見的侷限性及耗時性，已無法滿足快速進展且減少試誤性試驗及產業趨勢。例如常見之觀賞作物，如玫瑰花、菊花、康乃馨等，因其基因背景複雜，基因異質結合性高、染色體數量多、倍數性差異、生長週期長，以及包含部分自交不親和現象，如透過雜交育種方式，雖可從子代中選拔預期性狀，但目標性狀基因之遺傳無法推估，大大限制未來品種開發之親本選擇及時間。而就誘變育種的角度思考，其最大爭議在突變之隨機及不穩定性、往往需要花費大量人力時間進行測試適當誘變藥劑、時間等前置作業，而誘變株選拔也需要大量人力。另外誘變株也可能因為嵌合體現象，其植物特徵無法遺傳至後代。而基因轉殖透過外源目標基因之導入，可從其他物種轉移基因，增加了品種開發者對育種成品的想像空間。然而外來基因的隨機性插入，也可能造成原始植物體所具之功能性基因受到排擠、喪失功能，其不穩定性亦如前述，為其發展困境之原因。同時基因轉殖效率更容易受到物種及品種影響（Giovannini *et al.*, 2021），安全性評估仍受到許多國家質疑。目前僅開放花色改良品種可供於市場流通，如轉基因藍色康乃馨，以及在日本和美國銷售的藍色玫瑰（Kishi-Kaboshi *et al.*, 2018）、藍色菊花（Tanaka *et al.*, 2009）、藍色蝴蝶蘭（Mishiba *et al.*, 2005）。且限定於澳大利亞、加拿大、歐盟、日本、俄羅斯、阿拉伯聯合大公國和美國流通。因此開發一個精準且無外源基因介入之新興育種技術可解除基因轉殖之育種發展限制。

基因編輯技術，特別是那些基於（CRISPR-Cas）之衍生技術（Hahne *et al.*, 2019），能夠更加精準及高效，透過鎖定特定序列進行剪裁及黏貼，達到誘導植物基因突變，改變其表達或使其靜默現象。其概念如同自然界中，植物體基因序列發生突變之原理，基因編輯已應用在許多作物，如農藝作物、森林植物等。觀賞作物於近年方才蓬勃發展，因此極具研究潛力。其主要研究領域，主要針對花色（Boutigny *et al.*, 2020、Nishihara *et al.*, 2018、Nitarska *et al.*, 2021、Tanaka *et al.*, 2010、Watanabe *et al.*, 2017、Yan *et al.*, 2019）、大小、形狀、香味、抗病性、花朵壽命（Lin and Jones, 2022、Shibuya *et al.*, 2018、Xu *et al.*, 2020）等提升花卉品質為研究目的。作物種類包含：玫瑰花（Lin and Jones, 2022、Wang *et al.*, 2023、Xu *et al.*, 2020、Yu *et al.*, 2020）、朝顏（Shibuya *et al.*, 2018、Watanabe *et al.*, 2018）、夏堇（Nishihara *et al.*, 2018）、百合（Yan *et al.*, 2019）、菊花（Kishi-Kaboshi *et al.*, 2017、Su *et al.*, 2019）。由於蘭科作物於全球花卉產業之重要性，因此蘭科作物（Cardoso *et al.*, 2020、Semiarti *et al.*, 2020）目前也有許多物種進行相關研究如：台灣蝴蝶蘭（*Phalaenopsis aphrodite*）（Chao *et al.*, 2018）和姬蝴蝶蘭（*Phalaenopsis equestris*）（Cai *et al.*, 2015）、鐵皮石斛（*Dendrobium catenatum*）（Zhang *et al.*, 2016）、霍山石斛（*Dendrobium officinale*）（Yan *et al.*, 2015）及其他蘭科天麻屬（*Gastrodia elata*）（Yuan *et al.*, 2018）。透過基因編輯，可擴大花卉育種技術改良植物性狀並精準執行，唯基因編輯方式需透過全基因體之功能性序列解序、基因組層次、及建構於良好之組織培養技術及基因轉殖再生系統方能順利進行。



鑑於基因編輯技術已有成功案例及產業施行，如 2016 年美國農業部（USDA）核准利用 CRISPR-Cas9 系統成功編輯了蘑菇（*Agaricus bisporus*）的基因，美國決定不將其視為轉基因生物（GMO）（Waltz, 2018）。此外日本也即將通過 SDN-1 型修飾產生的基因組編輯終端產品，定義為非基因轉殖產物（Tsuda *et al.*, 2019）。如前所述，透過基因編輯 SDN-1 型修飾（不使用 DNA 範本的突變）產生的基因組編輯終端產品定義為不代表轉基因生物（Tsuda *et al.*, 2019）。基因編輯 SDN-1 型修飾其突變無外來基因插入，且因為瞭解全基因體基因序列及訊息而產生之精準位點變異，更可減少人力消耗及世代選拔之工作量。同植物體突變受到自然環境（逆境如強光、高溫、乾旱、放射線）、人為控制（藥劑化學誘變、放射性物理誘變），因而產生可供人類利用之園藝產物。在目前世界各國家對基因編輯作物或及開發之產品，在特定技術開發及管理下，給予開放且正面之趨勢，希冀台灣能夠在於潮流前，掌握關鍵技術。如蘭花為台灣花卉產業重要產品，相關學術研究支持也應於全球佈局之前進行，方能為未來產業發展搶得先機。因此若待全球已承認，可能已喪失掌握先機之時間點。因此，歸根究柢，以開放但嚴謹的態度，結合傳統育種和基因組編輯策略，將可開發安全且更有觀賞價值之花卉作物，提升人類的精神福祉。

■ 結語

隨著花卉育種技術的快速發展，本文所探討的新興育種策略和技術已經顯著改變了花卉產業的面貌。遠緣雜交、誘變育種、原生質體技術、基因轉殖和基因編輯等育種方法，各有其獨特的應用價值和挑戰，但共同的目標均是開發新的花卉品種以滿足市場需求。這些技術不僅加速了花卉的性狀改良，也提高了育種的靈活性和創新潛力。未來隨著基因體學、生物資訊、等分子輔助育種方式的進一步成熟和應用，預期將對花卉的外觀、抗逆性、生長周期等特性進行更細緻的調整。此外透過結合不同育種技術使用，將進一步提升育種效率，為全球花卉市場帶來更多具有創新特性的品種。最終這些先進育種技術不僅是科學進步的產物，更是推動花卉產業持續創新和增長的關鍵因素，有助於滿足不斷變化的消費者需求和提升美學追求。



■ 參考文獻

- 邱金春、王三太。2011。遠緣雜交育種障礙及克服策略。農業試驗所技術服務。22:19-22。
- 葉怡廷。2021。舞春花之屬間雜交及多倍體新品種育成。國立中興大學園藝學系碩士學位論文。pp. 32。
- 蔡奇助、翁一司。2014。狐狸尾蝶蘭品種選育現狀。高雄區農業專訊。87:12-13。
- Abe, T., H. Ichida, Y. Hayashi, R. Morita, Y. Shirakawa, K. Ishii, T. Sato, H. Saito, and Y. Okumoto. 2018. Ion beam mutagenesis an innovative and effective method for plant breeding and gene discovery. Intl. Symp. on Plant Mutation Breeding and Biotechnology. 41: 411-424
- Adedeji, O. S., A. H. Naing, and C. Kim. 2020. Protoplast isolation and shoot regeneration from protoplast-derived calli of Chrysanthemum cv. White ND. Plant Cell Tiss. Organ Cult. 141:571-581.
- Afkhami-Sarvestani, R., M. Serek, and T. Winkelmann. 2012. Protoplast isolation and culture from Streptocarpus, followed by fusion with Saintpaulia ionantha protoplasts. Europ. J. Hort. Sci. 77:249-260.
- Alonso, J. M. and J. R. Ecker. 2006. Moving forward in reverse: genetic technologies to enable genome-wide phenomic screens in Arabidopsis. Nat. Rev. Genet. 7:524-536.
- Amano, J., D. Nakazawa, S. Kuwayama, Y. Mizuta, H. Okuno, Y. Watanabe, T. Godo, D. S. Han, and M. Nakano. 2009. Intergeneric hybridization among colchicaceous ornamentals, Gloriosa spp., Littonia modesta and Sandersonia aurantiaca via ovule culture. Plant Biotechnol. 26:535-541.
- Amri-Tiliouine, W., M. Laouar, A. Abdelguerfi, J. Jankowicz-Cieslak, L. Jankuloski, and B. J. Till. 2018. Genetic variability induced by gamma rays and preliminary results of low-cost TILLING on M2 generation of Chickpea (Cicer arietinum L.). Front. Plant Sci. 9:1568.
- Andersson, M., H. Turesson, N. Olsson, A. S. Falt, P. Ohlsson, M. N. Gonzalez, M. Samuelsson, and P. Hofvander. 2018. Genome editing in potato via CRISPR-Cas9 ribonucleoprotein delivery. Physiol. Plant. 164:378-384.
- Azad, M. A. K., A. Begum, M. H. Rani, and M. Mirza. 2018a. Carbon ion beam irradiation technique shortens breeding cycle and induces novel mutants in rice. FAO/IAEA Intl. Symp. on Plant Mutation Breeding and Biotechnology.
- Azad, M. A. K., F. Yasmine, H. A. Begum, H. R. Md, and K. Md. 2018b. Development of



- climate change adaptable resistant crop varieties through nuclear techniques. FAO/IAEA Intl. Symp. on Plant Mutation Breeding and Biotechnology.
- Beal, J. M. and N. J. Scully. 1950. Chromosomal aberrations in onion roots from plants grown in an atmosphere containing C14 Q2. Bot. Gaz. 112:232-235.
- Bourzac, K. 2024. Bioluminescent houseplant hits US market for first time. Nature News. 626:701.
- Boutigny, A. L., N. Dohin, D. Pornin, and M. Rolland. 2020. Overview and detectability of the genetic modifications in ornamental plants. Hort. Res. 7:11.
- Bowen, J. M. and P. A. Cawse. 1962. The effects of deficiencies in essential elements on the radiosensitivity of tomato seeds. Radiat. Bot. 1:215-222.
- Cai, J., X. Liu, K. Vanneste, S. Proost, W. C. Tsai, K. W. Liu, L. J. Chen, Y. He, Q. Xu, C. Bian, Z. Zheng, F. Sun, W. Liu, Y. Y. Hsiao, Z. J. Pan, C. C. Hsu, Y. P. Yang, Y. C. Hsu, Y. C. Chuang, A. Dievart, J. F. Dufayard, X. Xu, J. Y. Wang, J. Wang, X. J. Xiao, X. M. Zhao, R. Du, G. Q. Zhang, M. Wang, Y. Y. Su, G. C. Xie, G. H. Liu, L. Q. Li, L. Q. Huang, Y. B. Luo, H. H. Chen, Y. Van de Peer, and Z. J. Liu. 2015. The genome sequence of the orchid *Phalaenopsis equestris*. Nat. Genet. 47:65-72.
- Caldwell, D. G., N. McCallum, P. Shaw, G. J. Muehlbauer, D. F. Marshall, and R. Waugh. 2004. A structured mutant population for forward and reverse genetics in Barley (*Hordeum vulgare* L.). Plant J. 40:143-150.
- Campbel, L. G. I, A. A. Snow, and P. M. Sweeney. 2009. When divergent life histories hybridize: insights into adaptive life-history traits in an annual weed. New Phytol. 184:806-818.
- Cao, X., H. Xie, M. Song, M. Lu, P. Ma, B. Huang, M. Wang, Y. Tian, F. Chen, J. Peng, Z. Lang, G. Li, and J. K. Zhu. 2022. Cut–dip–budding delivery system enables genetic modifications in plants without tissue culture. The Innovation. 4:100345.
- Cardoso, J. C., C. A. Zanello, and J. T. Chen. 2020. An overview of orchid protocorm-like bodies: mass propagation, biotechnology, molecular aspects, and breeding. Int. J. Mol. Sci. 21:985.
- Chadwick, K. H. and H. P. Leenbouts. 1981. The molecular theory of radiation biology. Springer, Berlin. pp. 377.
- Chao, Y. T., W. C. Chen, C. Y. Chen, H. Y. Ho, C. H. Yeh, Y. T. Kuo, C. L. Su, S. H. Yen, H. Y. Hsueh, J. H. Yeh, H. L. Hsu, Y. H. Tsai, T. Y. Kuo, S. B. Chang, K. Y. Chen, and M. C. Shin. 2018. Chromosome-level assembly, genetic and physical mapping of *Phalaenopsis aphrodite* genome provides new insights into species adaptation and



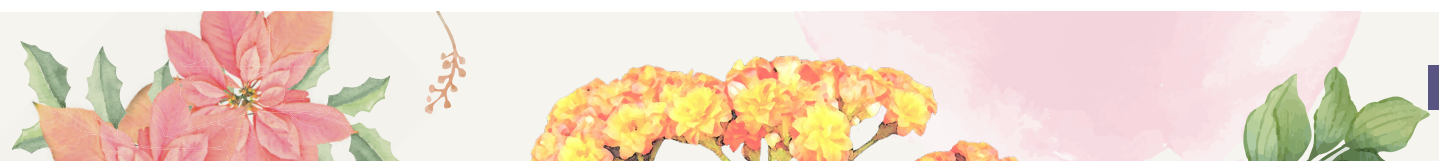
- resources for orchid breeding. *Plant Biotechnol.* 16:2027-2041.
- Chen, Y. M. and M. Mii. 2012. Inter-sectional hybrids obtained from reciprocal crosses between *Begonia semperflorens* (section *Begonia*) and B. 'Orange Rubra' (section *Gaerdita* X section *Pritzelia*). *Breed. Sci.* 62:113-123.
- Chen, Z. J. 2013. Genomic and epigenetic insights into the molecular bases of heterosis. *Nat. Rev. Genet.* 14:471-482.
- Colbert, T., B. J. Till, R. Tompa, S. Reynolds, M. N. Steine, A. T. Yeung, C. M. McCallum, L. Comai, and S. Henikoff. 2001. High-throughput screening for induced point mutations. *Plant Physiol.* 126:480-484.
- Comai, L. and S. Henikoff. 2006. Tilling: practical single-nucleotide mutation discovery. *Plant J.* 45:684-694.
- Comai, L., K. Young, B. J. Till, S. H. Reynolds, E. A. Greene, C. A. Codomo, L. C. Enns, J. E. Johnson, C. Burtner, A. R. Odden, and S. Henikoff. 2004. Efficient discovery of DNA polymorphisms in natural populations by Ecotilling. *Plant J.* 37:778-786.
- Cordeiro, G., F. G. Elliott, and R. J. Henry. 2006. An optimized ecotilling protocol for polyploids or pooled samples using a capillary electrophoresis system. *Anal. Biochem.* 355:145-147.
- Cronn, R. and J. F. Wendel. 2003. Cryptic trysts, genomic mergers, and plant speciation. *New Phytol.* 161:133-142.
- Cross, M. J., D. Waters, L. S. Lee, and R. J. Henry. 2008. Endonucleolytic mutation analysis by internal labeling (EMAIL). *Electrophoresis.* 29:1291-1301.
- Cui, J., K. Kuligowska Mackenzie, T. Eeckhaut, R. Muller, and H. Lutken. 2019. Protoplast isolation and culture from *Kalanchoe* species: optimization of plant growth regulator concentration for efficient callus production. *Plant Cell Tiss. Organ Cult.* 138:287-297.
- Datta, A. K. 2015a. Contribution to plant science a compilation since 1983. Bishnupriya Printers, Kalyani.
- Datta, S. K. 1989. Improvement of ornamental plants through induced mutation. In: Recent advances in genetics and cytogenetics. Premier Publishing House. pp. 215-228.
- Datta, S. K. 1992. Mutation studies on double bracted *Bougainvillea* at National Botanical Research Institute. *Mutation Breeding Newsletter.* 39: 8-9.
- Datta, S. K. 1994. Induction and analysis of somatic mutations in vegetatively propagated ornamentals. D.Sc. thesis. Kanpur University, Kanpur.
- Datta, S. K. 2000. Exploring the chrysanthemum in India. EBIS, NBRI, Lucknow.



- Datta, S. K. 2004. Bougainvillea research at National Botanical Research Institute, Lucknow. J. Ornamental Hort. 7:1-14.
- Datta, S. K. 2005a. Technological advances for development of new ornamental varieties. In: Advances in biotechnology. Agrobios (India), Jodhpur. pp. 285-304.
- Datta, S. K. 2005b. Role of classical mutaiton breeding in crop improvement. Daya Publishing House, Delhi. pp. 1-314.
- Datta, S. K. 2009a. Role of classical mutagenesis for development of new ornamental varieties. In: Induced plant mutations in the genomics era. FAO, Vienna. pp. 300-302.
- Datta, S. K. 2009b. A report on 36 years of practical work on crop improvement through induced mutagenesis. In: Induced plant mutations in the genomics era. FAO, Vienna. pp. 253-256.
- Datta, S. K. 2009c. Plant chimeras and their role in development of new ornamental varieties. J. Ornamental Hort. 12:75-94.
- Datta, S. K. 2015b. Indian floriculture: role of CSIR. Regency Publications, New Delhi. p. 432.
- Datta, S. K. 1988. Chrysanthemum cultivars evolved by induced mutations at National Botanical Research Institute, Lucknow. Chrysanthemum. 44:72-75.
- Datta, S. K. 1997. Ornamental plants and role of mutation. Daya Publishing House, Delhi. p. 220.
- Datta, S. K. 2023. Introduction. In: Induced Mutation Breeding. Springer Nature Singapore Pte Ltd. pp. 1-73.
- Davis, D. R. and E. T. Wall. 1961. Induced mutations at the Vby locus of Trifolium repens II. Reduction below the additive base line by fractionated doses of gamma radiation. Genetics. 46:787-798.
- Duarte, P., D. Ribeiro, I. Carqueijeiro, S. Bettencourt, and M. Sottomayor. 2016. Protoplast transformation as a plant-transferable transient expression system. Biotechnol. Plant Second. Metab. 1405:137-148.
- Elliott, F., G. Cordeiro, and P. C. Bundock. 2008. SNP discovery by ECOTILLING using capillary electrophoresis. In: Plant Genotyping 2. CABI, London, UK. 5:78-87.
- English, C. and K. Schreiber. 2020. Where are GMO crops grown? GLP infographics document the global growth of agricultural biotechnology innovation. Genetic Literacy Project.
- Etter, L. E. 1965. The science of ionizing radiations: modes of application. Springfield III. p.



- 788.
- Evans, H. J. 1962. Chromosome aberrations induced by ionizing radiations. *Int. Rev. Cytol.* 13:221-321.
- Evans, H. J. 1966. Repair and recovery from chromosome damage after fractionated x-ray dosage. In: *Genetical aspects of radiosensitivity: mechanisms of repair* (Proc. Panel Vienna), IAEA, Vienna. pp. 31-48.
- Ford, C. E. 1948. Chromosome breakage on nitrogen mustard treated *Vicia faba* root tip cells. *Proc. 8th Int. Cong. Genetics, Lund.* p. 570.
- Friesleben, R. A. and A. Lein. 1942. Plant mutagenesis in crop improvement basic terms and applications. In: *Plant mutation breeding and biotechnology.* pp 9–15.
- Furuta, H., H. Shinoyama, Y. Nomura, M. Maeda, and K. Makara. 2004. Production of intergeneric somatic hybrids of chrysanthemum [*Dendranthema* × *grandiflorum* (Ramat.) Kitamura] and wormwood (*Artemisia sieversiana* J. F. Ehrh. ex. Willd) with rust (*Puccinia horiana* Henning) resistance by electrofusion of protoplasts. *Plant Sci.* 166:695-702.
- Gilchrist, E. J., G. W. Haughn, C. C. Ying, S. P. Otto, J. Zhuang, D. Cheung, B. Hamberger, F. Aboutorabi, T. Kalynyak, L. Johnson, J. Bohlmann, B. E. Ellis, C. J. Douglas, and Q. C. B. Cronk. 2006a. Use of Ecotilling as an efficient SNP discovery tool to survey genetic variation in wild populations of *Populus trichocarpa*. *Mol. Ecol.* 15:1367-1378.
- Gilchrist, Erin. J., N. J. O'Neil, A. M. Rose, M. C. Zetka, and G. W. Haughn. 2006b. TILLING is an effective reverse genetics technique for *Caenorhabditis elegans*. *BMC Genomics.* 7:262.
- Giovannini, A., M. Laura, B. Nesi, M. Savona, and T. Cardi. 2021. Genes and genome editing tools for breeding desirable phenotypes in ornamentals. *Plant Cell Rep.* 40: 461-478.
- Giraud, T., G. Refre' gier, M. L. Gac, D. M. de Vienne, and M. E. Hood. 2008. Speciation in fungi. *Fungal Genet. Biol.* 45:791-802.
- Gompert, Z., J. A. Fordyce, M. L. Forister, A. M. Shapiro, and C. C. Nice. 2006. Homoploid hybrid speciation in an extreme habitat. *Science.* 314:1923-1925.
- Gross, B. L. and L. H. Rieseberg. 2005. The ecological genetics of homoploid hybrid speciation. *J. Hered.* 96:241-252.
- Gunckel, J. E. 1957. The effects of ionizing radiation on plants. Morphological effects. In: *Symp. effects of ionizing radiation on plants.* Q. Rev. Biol. 32:46-56.
- Gustafsson, Å. 1947a. Mutations in agricultural plants. *Hereditas.* 24:3-93.



- Gustafsson, Å. 1947b. Mutations in agricultural plants. *Hereditas*. 33:1-100.
- Hahne, G., L. Tomlinson, and F. Nogué. 2019. Precision genetic engineering tools for next-generation plant breeding. *Plant Cell Rep.* 38:435-436.
- Hawkins, S. M., J. M. Ruter, and C. D. Robacker. 2016. Interspecific and intergeneric hybridization in *Dissotis* and *Tibouchina*. *HortScience*. 51:325-329.
- Henikoff, S. and L. Coma. 2003. Single-nucleotide mutations for plant functional genomics. *Annu. Rev. Plant Biol.* 54:375-401.
- Hoffmann, W. 1959. Neuere möglichkeiten der mutationszuchtung. *Z Pflanzenz.* 41:371-394.
- Horita, M., H. Morohashi, and F. Komai. 2003. Production of fertile somatic hybrid plants between Oriental hybrid lily and *Lilium* × *formolongi*. *Planta* 217:597-601.
- Jitsopakul, N., A. Chunthaworn, U. Pongket, and K. Thammasiri. 2022. Interspecific and intergeneric hybrids of *Aerides* species with *Rhynchostylis coelestis* Rchb.f. and germination of hybrid seeds in vitro. *Trends Sci.* 19:3429.
- Kalisz, S. and E. M. Kramer. 2008. Variation and constraint in plant evolution and development. *Heredity*. 100:171-177.
- Kang, H. H., A. H. Naing, and C. K. Kim. 2020. Protoplast isolation and shoot regeneration from protoplast-derived callus of *Petunia hybrida* Cv. Mirage Rose. *Biology* 9:228.
- Kästner, U., E. Klocke, and S. Abel. 2017. Regeneration of protoplasts after somatic hybridisation of *Hydrangea*. *Plant Cell Tiss. Organ Cult.* 129:359-373.
- Keshavareddy, G., A. R. V. Kumar, and V. S. Ramu. 2018. Methods of plant transformation- A review. *Int. J. Curr. Microbiol. App. Sci.* 7:2656-2668
- Kihlman, B. A. 1966. Actions of chemicals on dividing cells. Prentice Hall, Englewood Cliffs. p. 260.
- Kishi-Kaboshi, M., R. Aida, and K. Sasaki. 2017. Generation of gene-edited *Chrysanthemum morifolium* using multicopy transgenes as targets and markers. *Plant Cell Physiol.* 58:216-226.
- Kishi-Kaboshi, M., R. Aida, and K. Sasaki. 2018. Genome engineering in ornamental plants: current status and future prospects. *Plant Physiol. Biochem.* 131:47-52.
- Klein, T. M. 2011. Particle bombardment: An established weapon in the arsenal of plant biotechnologists. In *Plant Transformation Technologies*. Stewart, C. N., Blackwell Publishing Ltd. pp. 53-71.
- Knobloch, I. W. 1972. Intergeneric hybridization in flowering plants. *Taxon*. 21:97-103.
- Kölreuter, J. G. 1766. Vorläufige nachricht von einigen das geschlecht der pflanzen



- betreffenden versuchen und beobachtungen, nebst for tsetzungen. Wilhelm Engelmann, Leipzig, Germany. pp. 1761-1766.
- Laouar, M., D. Tabti, W. Tiliouine, J. Jankowicz, and B. J. Till. 2018. Early assessment of lentil and chickpea mutant and evaluation of low cost TILLING on M2 chickpea. Intl. Symp. on Plant Mutation Breed. and Biotech.
- Lee, J. W., J. K. Kim, P. Srinivasan, J. Choi, J. H. Kim, S. B. Han, D. Kim, and M. W. Byun. 2009a. Effect of gamma irradiation on microbial analysis, antioxidant activity, sugar content and color of ready-to-use tamarind juice during storage. LWT- Food Sci. Technol. 42:101-105.
- Lee, L. S., M. J. Cross, and R. J. Henry. 2009b. EMAIL - a highly sensitive tool for specific mutation detection in plant improvement programs. In: Induced plant mutations in the genomics era. FAO of the United Nations, Rome. pp. 243-244.
- Lin, Y. and M. L. Jones. 2022. CRISPR/Cas9-Mediated Editing of Autophagy Gene 6 in petunia decreases flower longevity, seed yield, and phosphorus remobilization by accelerating ethylene production and senescence-related gene expression. Front. Plant Sci. 13:840218.
- MacKey, J. 1956. Mutation breeding in Europe. In: Genetics in plant breeding. Brookhaven. Symp. in Biol. 9:141-152.
- Mallet, J. 2007. Hybrid speciation. Nature. 446:279-283.
- Mavárez, J. and M. Linares. 2008. Homoploid hybrid speciation in animals. Mol. Ecol. 17:4181-4185.
- Mejlhede, N., Z. Kyjovska, G. Backes, K. Burhenne, S. K. Rasmussen, and A. Jahoor. 2006. EcoTILLING for the identification of allelic variation in the powdery mildew resistance genes mlo and Mla of barley. Plant Breed. 125:461-467.
- Meyer, L., M. Serek, and T. Winkelmann. 2009. Protoplast isolation and plant regeneration of different genotypes of Petunia and Calibrachoa. Plant Cell Tiss. Organ Cult. 99:27-34.
- Mii, E. M. and D. P. Chin. 2024. This Japanese orchid just got a glow up, literally. Tokyo Weekender
- Mishiba, K., D. P. Chin, and M. Mii. 2005. Agrobacterium-mediated transformation of Phalaenopsis by targeting protocorms at an early stage after germination. Plant Cell Rep. 24:297-303.
- Murovec, J., K. Gušek, B. Bohanec, M. Avbelj, and R. Jerala. 2018. DNA-free genome editing of Brassica oleracea and B. rapa protoplasts using CRISPR-Cas9



- ribonucleoprotein complexes. *Front. Plant Sci.* 9:1594.
- Naing, A. H., O. S. Adedeji, and C. K. Kim. 2021. Protoplast technology in ornamental plants: Current progress and potential applications on genetic improvement. *Sci. Hortic.* 283:110043.
- Nakano, M. and M. Mii. 1993. Somatic hybridization between *Dianthus chinensis* and *D. barbatus* through protoplast fusion. *Theor. Appl. Genet.* 86:1-5.
- Nakano, M., Y. Hoshino, and M. Mii. 1996. Intergeneric somatic hybrid plantlets between *Dianthus barbatus* and *Gypsophila paniculata* obtained by electrofusion. *Theor. Appl. Genet.* 92:170-172.
- Nakano, M., Y. Hoshino, and M. Mii. 1996. Intergeneric somatic hybrid plantlets between *Dianthus barbatus* and *Gypsophila paniculata* obtained by electrofusion. *Theor. Appl. Genet.* 92:170-172.
- Nassour, M. and N. Dorion. 2002. Plant regeneration from protoplasts of micropropagated *Pelargonium x hortorum* 'Alain': effect of some environmental and medium factors on protoplast system efficiency. *Plant Sci.* 163:169-176.
- Nieto, C., F. Piron, M. Dalmais, C. F. Marco, E. Moriones, M. L. Gómez-Guillamón, V. Truniger, P. Gómez, J. Garcia-Mas, M. A. Aranda, and A. Bendahmane. 2007. EcoTILLING for the identification of allelic variants of melon eIF4E, a factor that controls virus susceptibility. *BMC Plant Biol.* 7:34.
- Nilan, R. A., C. F. Konzak, J. Wagner, and R. R. Legault. 1965. Effectiveness and efficiency of radiations for inducing genetic and cytogenetic changes. In: *The use of induced mutations in plant breeding*. Pergamon Press. pp. 71-89.
- Nishihara, M., A. Higuchi, A. Watanabe, and K. Tasaki. 2018. Application of the CRISPR/Cas9 system for modification of flower color in *Torenia fournieri*. *BMC Plant Biol.* 18:331.
- Nitarska, D., R. Boehm, T. Debener, R. C. Lucaciu, and H. Halbworth. 2021. First genome edited poinsettias: targeted mutagenesis of flavonoid 3'-hydroxylase using CRISPR/Cas9 results in a colour shift. *Plant Cell Tiss. Organ cult.* 147: 49-60.
- Noda, N. 2018. Recent advances in the research and development of blue flowers. *Breed. Sci.* 68:79-87
- O' Brien, S. J. 1983. Miami Winter Symposium. *Nat. Biotechnol.* 1:36-38
- Oleykowski, C. A., C. R. B. Mullins, A. K. Godwin, and A. T. Yeung. 1998. Mutation detection using a novel plant endonuclease. *Nucleic Acids Res.* 26:4597-4602.



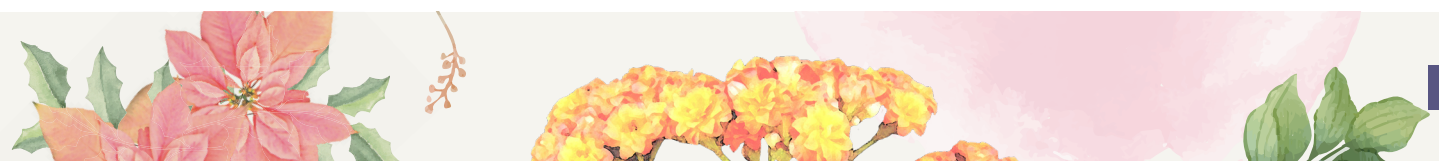
- Ostergaard, L. and M. Yanofsky. 2004. Establishing gene function by mutagenesis in *Arabidopsis thaliana*. *Plant J.* 39:682-696.
- Pati, P. K., M. Sharma, and P. S. Ahuja. 2008. Rose protoplast isolation and culture and heterokaryon selection by immobilization in extra thin alginate film. *Protoplasma* 233:165-171.
- Paun, O., F. Forest, M. F. Fay, and M. W. Chase. 2009. Hybrid speciation in angiosperms: parental divergence drives ploidy. *New Phytol.* 182:507-518.
- Power, J. B., S. F. Berry, J. V. Chapman, and E. C. Cocking. 1980. Somatic hybridization of sexually incompatible *Petunias*: *Petunia parodii*, *Petunia parviflora*. *Theor. Appl. Genet.* 57:1-4.
- Rao, A. Q., A. S. B. Kiani, K. Shahzad, A. A. Shahid, T. Husnain, and S. Riazuddin. 2009. The Myth of Plant Transformation. *Biotechnol.* 27:753-763
- Rieseberg, L. H., O. Raymond, D. M. Rosenthal, Z. Lai, K. Livingstone, T. Nakazato, J. L. Durphy, A. E. Schwarzbach, L. A. Donovan, and C. Lexer. 2003. Major ecological transitions in wild sunflowers facilitated by hybridization. *Science.* 301:1211-1216.
- Sato, Y., K. Shirasawa, Y. Takahashi, M. Nishimura, and T. Nishio. 2006. Mutant selection from progeny of gamma-ray irradiated rice by DNA heteroduplex cleavage using *Brassica* petiole extract. *Breed. Sci.* 56:179-183.
- Schwenk, K., N. Brede, and B. Streit. Introduction. Extent, processes and evolutionary impact of interspecific hybridization in animals. *Phil. Trans. R. Soc. B.* 363:2805-2811.
- Semiarti, E., S. Nopitasari, Y. Setiawati, M. D. Lawrie, A. Purwantoro, J. Widada, K. Ninomiya, Y. Asano, S. Matsumoto, and Y. Yoshioka. 2020. Application of CRISPR/Cas9 genome editing system for molecular breeding of orchids. *Indones. J. Biotechnol.* 25: 61-68.
- Shang, L., Y. Wang, S. Cai, X. Wang, Y. Li, A. Abduweli, and J. Hua. 2015. Partial dominance, overdominance, epistasis and QTL by environment interactions contribute to heterosis in two upland cotton hybrids. *G3-genes Genom. Genet.* 6:499-507.
- Shen, G., W. Zhan, H. Chen, and Y. Xing. 2014. Dominance and epistasis are the main contributors to heterosis for plant height in rice. *Plant Sci.* 215-216:11-18.
- Shen, Y., D. Meng, K. McGrouther, J. Zhang, and L. Cheng. 2017. Efficient isolation of *Magnolia* protoplasts and the application to subcellular localization of MdeHSF1. *Plant Methods* 13:1-10.
- Shibuya, K., K. Watanabe, and M. Ono. 2018. CRISPR/Cas9-mediated mutagenesis of the



- EPHEMERAL1 locus that regulates petal senescence in Japanese morning glory. *Plant Physiol. Biochem.* 131:53-57.
- Slade, A. J. and V. C. Knauf. 2005. TILLING moves beyond functional genomics into crop improvement. *Transgenic Res.* 14:109-115.
- Soltis, P. S. and D. E. Soltis. 2009. The role of hybridization in plant speciation. *Annu. Rev. Plant Biol.* 60:561-588.
- Stadler, L. J. 1928a. Mutations in barley induced by x-rays and radium. *Science* 68:186-187.
- Stadler, L. J. 1928b. Genetic effects of x-rays on maize. *Proc. Natl. Acad. Sci. U. S. A.* 14:69-75.
- Stebbins, G. L. 1959. The role of hybridization in evolution. *Proc. Amer. Phil. Soc.* 103:231-251.
- Su, J., J. Jiang, F. Zhang, Y. Liu, L. Ding, S. Chen, and F. Chen. 2019. Current achievements and future prospects in the genetic breeding of chrysanthemum: a review. *Hortic. Res.* 6:109.
- Subburaj, S., S. J. Chung, C. Lee, S. M. Ryu, D. H. Kim, J. S. Kim, S. Bae, and G. J. Lee. 2016. Site-directed mutagenesis in *Petunia* × *hybrida* protoplast system using direct delivery of purified recombinant Cas9 ribonucleoproteins. *Plant Cell Rep.* 35:1535-1544.
- Svitashev, S., C. Schwartz, B. Lenderts, J. K. Young, and A. M. Cigan. 2016. Genome editing in maize directed by CRISPR-Cas9 ribonucleoprotein complexes. *Nat. Commun.* 7:13274.
- Tanaka, Y. and F. Brugliera. 2013. Flower colour and cytochromes P450. *Phil. Trans. R. Soc. B.* 368:1612
- Tanaka, Y., F. Brugliera, and S. Chandler. 2009. Recent progress of flower colour modification by biotechnology. *Int. J. Mol. Sci.* 10:5350-5369.
- Tanaka, Y., F. Brugliera, G. Kalc, M. Senior, B. Dyson, N. Nakamura, Y. Katsumoto, and S. Chandler. 2010. Flower color modification by engineering of the flavonoid biosynthetic pathway: Practical perspectives. *Biosci. Biotechnol. Biochem.* 74:1760-1769.
- Tang, J., J. Yan, X. Ma, W. Teng, W. Wu, J. Dai, B. S. Dhillon, A. E. Melchinger, and J. Li. 2010. Dissection of the genetic basis of heterosis in an elite maize hybrid by QTL mapping in an immortalized F2 population. *Theor. Appl. Genet.* 120:333-340.
- Till, B. J., J. Jankowicz-Cieslak, L. Sa' gi, O. A. Huynh, H. Utsushi, R. Swennen, R.



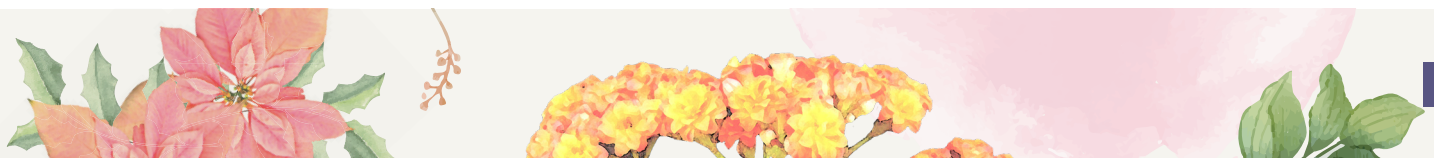
- Terauchi, and C. Mba. 2010. Discovery of nucleotide polymorphisms in the *Musa* gene pool by Ecotilling. *Theor. Appl. Genet.* 121:1381-1389.
- Tomiczak, K. 2020. Molecular and cytogenetic description of somatic hybrids between *Gentiana cruciata* L. and *G. tibetica* King. *J. Appl. Genet.* 61:13-24.
- Tomiczak, K., E. Sliwinska, and J. J. Rybczyński. 2016. Comparison of the morphogenic potential of five *Gentiana* species in leaf mesophyll protoplast culture and ploidy stability of regenerated calli and plants. *Plant Cell Tiss. Organ Cult.* 126:319-331.
- Tomiczak, K., E. Sliwinska, and J. J. Rybczyński. 2017. Protoplast fusion in the genus *Gentiana*: genomic composition and genetic stability of somatic hybrids between *Gentiana kurroo* Royle and *G. cruciata* L. *Plant Cell Tiss. Organ Cult.* 131:1-14.
- Tsuda, M., K. Watanabe, and R. Ohsawa. 2019. Regulatory status of genome-edited organisms under the Japanese Cartagena act. *Front. Bioeng. Biotechnol.* 7:387.
- Waltz, E. 2018. With a free pass, CRISPR-edited plants reach market in record time. *Nat. Biotechnol.* 36:6-7.
- Wang, C., Y. Li, N. Wang, Q. Yu, Y. Li, J. Gao, X. Zhou, and N. Ma. 2023. An efficient CRISPR/Cas9 platform for targeted genome editing in rose (*Rosa hybrida*). *J. Integr. Plant Biol.* 65:895-899.
- Wang, H. C., S. M. Qiu, J. S. Zheng, L. R. Jiang, H. Z. Huang, and Y. M. Hung. 2009b. Generation of new rice cultivars from mature pollen treated with gamma-radiation. In: *Induced plant mutations in the genomic era*. FAO of the United Nations, Rome. pp. 231-234.
- Wang, T. L., J. Perry, T. Welham, J. Pike, M. Parniske, C. Rogers, G. E. D. Oldroyd, P. Stephenson, L. Ostergaard, B. McCullagh, D. Baker, S. Walsh, and J. Clarke. 2009a. Driving forward in reverse. In: *Induced plant mutations in the genomics era*. FAO of the United Nations, Rome. pp. 247-250.
- Watanabe, K. A. Kobayashi, M. Endo, K. Sage-Ono, S. Toki, and M. Ono. 2017. CRISPR/Cas9-mediated mutagenesis of the dihydroflavonol-4-reductase-B (DFR-B) locus in the Japanese morning glory *Ipomoea (Pharbitis) nil*. *Sci. Rep.* 7:10028.
- Watanabe, K., C. Oda-Yamamizo, K. Sage-Ono, A. Ohmiya, M. Ono. 2018. Alteration of flower colour in *Ipomoea nil* through CRISPR/Cas9-mediated mutagenesis of carotenoid cleavage dioxygenase 4. *Transgenic Res.* 27:25-38.
- Whitney, K. D., R. A. Randell, and L. H. Rieseberg. 2006. Adaptive introgression of herbivore resistance traits in the weedy sunflower *Helianthus annuus*. *Am. Nat.* 167:794-807.



- Whitney, K. D., R. A. Randell, and L. H. Rieseberg. 2010. Adaptive introgression of abiotic tolerance traits in the sunflower *Helianthus annuus*. *New Phytol.* 187:230-239.
- Xia, K., D. Zhang, G. Liu, X. Xu, Y. Yang, G. Q. Zhang, H. X. Sun, and Y. Gu. 2020. Efficient multiplex genome editing tools identified by protoplast technology in *Phalaenopsis*. *BioRxiv*. <https://doi.org/10.1101/2020.09.29.315200>.
- Xu, J., B. C. Kang, A. H. Naing, S. J. Bae, J. S. Kim, H. Kim, and C. K. Kim. 2020. CRISPR/Cas9-mediated editing of 1-aminocyclopropane-1-carboxylate oxidase1 enhances *Petunia* flower longevity. *Plant Biotechnol.* 18:287-297.
- Yan, L., X. Wang, H. Liu, Y. Tian, J. Lian, R. Yang, S. Hao, X. Wang, S. Yang, Q. Li, S. Qi, L. Kui, M. Okpekum, X. Ma, J. Zhang, Z. Ding, G. Zhang, W. Wang, Y. Dong, and J. Sheng. 2015. The genome of *Dendrobium officinale* illuminates the biology of the important traditional Chinese orchid herb. *Mol. Plant* 8:922-934.
- Yan, R., Z. Wang, Y. Ren, H. Li, N. Liu, and H. Sun. 2019. Establishment of efficient genetic transformation systems and application of CRISPR/Cas9 genome editing technology in *Lilium pumilum* DC. Fisch. and *Lilium longiflorum* White Heaven. *Int. J. Mol. Sci.* 20:2920.
- Yu, J., L. Tu, S. Subburaj, S. Bae, and G. J. Lee. 2021. Simultaneous targeting of duplicated genes in *Petunia* protoplasts for flower color modification via CRISPR-Cas9 ribonucleoproteins. *Plant Cell Rep.* 40:1037-1045.
- Yu, J., L. Tu, S. Subburaj, S. Bae, and G. J. Lee. 2020. Simultaneous targeting of duplicated genes in *Petunia* protoplasts for flower color modification via CRISPR Cas9 ribonucleoprotein. *Plant Cell Rep.* 21:1037-1045.
- Yuan, Y., X. Jin, J. Liu, X. Zhao, J. Zhou, X. Wang, D. Wang, C. Lai, W. Xu, J. Huang, L. Zha, D. Liu, X. Ma, L. Wang, M. Zhou, Z. Jiang, H. Meng, H. Peng, Y. Liang, R. Li, C. Jiang, Y. Zhao, T. Nan, Y. Jin, Z. Zhan, J. Yang, W. Jiang, and L. Huang. 2018. The *Gastrodia elata* genome provides insights into plant adaptation to heterotrophy. *Nat. Commun.* 9:1615.
- Zhang, G. Q., Q. Xu, C. Bian, W. C. Tsai, C. M. Yeh, K. W. Liu, K. Yoshida, L. S. Zhang, S. B. Chang, F. Chen, Y. Shi, Y. Y. Su, Y. Q. Zhang, L. J. Chen, Y. Yin, M. Lin, H. Huang, H. Deng, Z. W. Wang, S. L. Zhu, X. Zhao, C. Deng, S. C. Niu, J. Huang, M. Wang, G. H. Liu, H. J. Yang, X. J. Xiao, Y. Y. Hsiao, W. L. Wu, Y. Y. Chen, N. Mitsuda, M. Ohme-Takagi, Y. B. Luo, Y. Van de Peer, and Z. J. Liu. 2016. The *Dendrobium catenatum* Lindl. genome sequence provides insights into polysaccharide synthase, floral development and adaptive evolution. *Sci. Rep.* 12:19029.



Zhou, G., Y. Chen, W. Yao, C. Zhang, W. Xie, J. Hua, Y. Xing, J. Xiao, and Q. Zhang. 2012. Genetic composition of yield heterosis in an elite rice hybrid. *Proc. Nat. Acad. Sci.* 109:15847-15852.



Breeding Strategies and Technical Applications for Emerging Flower Cultivars

Pei-Jung Wen, Rou-Wei Lee, Yen-Ming Chen

National Chung Hsing University Department of Horticulture

■ Abstract

Present report discusses emerging strategies for breeding new flower cultivar, focusing on the application of breeding technologies such as distant hybridization, mutation breeding, protoplast technology, gene transformation and gene editing. Distant hybridization allows for the crossing of species boundaries to create new varieties with unique adaptability and biodiversity. Mutation breeding utilizes radiation or chemical methods to induce genetic variations, rapidly expanding the range of plant traits. Protoplast technology further breaks through reproductive barriers to develop entirely new floral varieties. Gene transfer and gene editing technologies assist breeders in precisely controlling plant genes, optimizing floral traits such as flower color, fragrance, and disease resistance. This article will delineate the evolution and development of traditional and modern breeding technologies, showcase recent achievements in the breeding of new flower varieties, and explain how these technologies and their outcomes are driving innovation in the flower industry.

Keywords: Distant hybridization, Mutation breeding, Protoplast, Gene transformation, Gene editing