抗嘉磷塞作物之發展及其抗藥性機制

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摘　要

嘉磷塞(glyphosate)為全球使用最多之非選擇性除草劑，其主要作用原理為抑制芳香族胺基酸生合成路徑中 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS)酵素之作用。由於此藥劑於雜草防除具上廣效性、價格低廉及對哺乳類動物低毒性的特質，於近十年來嘉磷塞的抗性常被應用於基因改造作物的研發。目前有三種途徑可增加作物對於嘉磷塞的抗性，最初之研究採用細胞培養方式，逐步增加嘉磷塞的濃度，篩選出抗性細胞株，主要原因為抗性細胞株的 EPSPS 基因增幅，EPSPS 酵素大量形成，或是增加酵素的穩定性之故。其次是利用源自土壤微生物可代謝嘉磷塞功能的酵素蛋白基因，包括編碼為 glyphosate oxidoreductase (GOX)或 glyphosate N-acetyl transferase (GAT)的轉基因，可避免嘉磷塞的傷害。另一方式為轉殖一具抗性 EPSPS 基因，其中來自農桿菌 CP4 菌株 (*Agrobacterium* sp. Strain CP4) 之 EPSPS 的抗性最強，目前商業化生產的抗嘉磷塞基改作物，其轉基因以 CP4 EPSPS 為主。

關鍵詞：嘉磷塞、基改作物、抗藥性、作用機制

Development of glyphosate-resistant crops and its resistance mechanism

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Abstract

Glyphosate (N-phophonomethyl glycine) is a widely used herbicide that acts non-selectively through inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the aromatic biosynthesis pathway. This low cost herbicide is environmental safe and can effectly control most weeds. In the past two decades, there are intensive researches on creating crops with glyphosate tolerance. The following 3 strategies have been used: progressive adaptation of cultured plant cells to increased glyphosate concentrations, transforming plants with glyphosate metabolism genes, insertion of a glyphosate tolerant EPSPS gene. This article present detail information on the strategies used on developing glyphosate tolerant crops.

Key words: glyphosate, genetically modified crop, herbicide-resistance, mechanism

嘉磷塞藥劑之特性及嘉磷塞抗性作物的發展

嘉磷塞為孟山都公司開發的除草劑，為有機磷類藥劑，屬於非選擇性系統型萌後除草劑，嘉磷塞之主要作用位置為 shikimic acid 代謝路徑中 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)酵素，嘉磷塞與受質 phosphoenolpyruvate (PEP) 產生競爭性之抑制，造成苯丙胺酸 (phenylalanine)、酪胺酸 (tyrosine) 及色胺酸 (tryptophan) 三種芳香族胺基酸含量降低，影響蛋白質之生合成，進而抑制植物生長，達成防除雜草之目的 (Kishore and Shah 1988)。由於嘉磷塞具有非選擇及廣效的特質，可有效防治大部份一年生及多年生雜草。此外嘉磷塞容易快速與土壤團粒結合，不易被雨淋洗至地下水，且可經由微生物分解，以及對哺乳類、鳥類及魚類低毒性等特點(Malik *et al*. 1989)，因此適合研發出抗此種除草劑之作物。。

全球抗除草劑作物栽種面積約為 7 仟萬公頃，以除草劑以嘉磷塞為主，其次為固殺草(glufosinate) (Global Knowledge Center on Crop Biotechnology 2007. (http://www.isaaa.org/kc/)。已通過登記而允許栽種的抗嘉磷塞作物有大豆(*Glycine max* L.)、玉米(*Zea mays* L.)、油菜(*Brassica napus* L.)、棉花(*Gossypiμm herbaceμm* L,)、甜菜(*Beta vulgaris* L.)、苜蓿(*Medicago sativa* L.)、小麥(*Triticum aestivum* L.)及翦股穎草(*Agrostis stolonifera* L.)等 8 類作物 33 個

品系(GM Database from AGBIOS, 2007)，各品系的抗性機制、轉基因的構築、編碼產物不盡相同，亦有同時抗二種除草劑及含其他功能的品系，8 種作物皆有嘉磷塞的單抗品系，如大豆 GST40-3-2 品系、玉米 GA21 及 NK603 品系，玉米及棉花有抗嘉磷塞及抗蟲的雙抗品系，如玉米 MON802 品系、棉花 MON15985 x MON88913 品系，或是同時可抗嘉磷塞、固殺草及抗蟲的三抗品系，如大豆 DAS-59122-7 x TC1507 x NK603 品系，此類品系多由不同特性的單抗品系，以傳統雜交育種後，再選拔出的品系(GM Database from AGBIOS, 2007)。

目前產生嘉磷塞抗性作物的方式有如後三類：(1)利用組織培養，進行重覆施藥，篩選出抗藥性品系。(2)基因轉殖：選殖抗性基因或修飾基因為具抗藥特性，轉殖於作物中，形成抗嘉磷塞品系。(3)不同特性基改作物品系間的雜交種選拔：利用具抗嘉磷塞品系與抗固殺草(glufosinate)或抗蟲品系進行雜交，選拔為具雙抗或三抗的新品系。其中主要運用的抗性機制可區分為三種：(1)大量產生 EPSPS 酵素：經由大量表現 EPSPS 基因，降低 EPSPS turnover 速率或是增幅 EPSPS 基因。(2)降低 EPSPS 酵素與嘉磷塞的親和性。(3)轉殖可代謝嘉磷塞功能的酵素蛋白基因。以下針對抗嘉磷塞作物的研發歷程及抗藥特性，分別詳述之。

利用組織培養篩選抗嘉磷塞植物

於 1980 至 1990 年間，多數研究利用組織培養時添加嘉磷塞，篩選具耐抗性植物。曾測試之植物包括紫堇科植物 rock harlequin (*Corydalis simpervirens* (L.) Pers.) 、野生胡蘿蔔(*Daucus carota* L.)、矮牽牛(*Petunia hybridus*)、長春花(*Catharanthus roseus* (L.) G. Don)、番茄(*Lycopersicon esculentum* Mill.)、菸草(*Nicotiana tabacum* L.)、苜蓿、大豆及玉米等(Table 1)。

最初報導的紫堇科植物（rock harlequin），於 10 mM 嘉磷塞的培養基繁殖 9 個月後，EPSPS 活性增加 40 倍(Holländer-Czytko *et al*. 1992)，但會累積大量的 shikimic acid (Amrhein *et al*.1983)，因為其 EPSPS 酵素為嘉磷塞敏感型，且 EPSPS 基因套數不變，其耐受性主要由是轉錄速率(transcription rate)增加，以及減緩 EPSPS 降解速率之故(Smart *et al*. 1985)，此抗性機制未見於其他植物或細胞株。野生胡蘿蔔細胞株經逐漸增加 24-35 mM 嘉磷塞藥劑濃度，可篩選出敏感型 EPSPS 增幅的細胞株(Murata *et al*. 1998)，此細胞株的 EPSPS 基因套數由 4 增為 25，且 EPSPS 活性增加 12 倍(Suh *et al*. 1993)。此外，矮牽牛篩選的細胞株可耐 1 mM 嘉磷塞，其 EPSPS 活性增加 20 倍

(Steinrucken *et al*. 1986)。大豆、苜蓿及菸草皆可經由藥劑處理，篩選出耐性細胞株，EPSPS 活性分別增加 21、62 及 80-10,000 倍，具有抗 35 mM 嘉磷塞之特性，主要原因為大量增幅敏感型 EPSPS (Widholm *et al*. 2001)。菊苣(*Cichorium intybus* L.)篩選的細胞株可耐 5 mM 嘉磷塞，其 EPSPS 活性增加 4.4 倍，但種子繁殖的子代則不具耐性 (Sellin *et al*. 1992)。玉米篩選出同時具敏感及耐受性 EPSPS 的細胞株，可抗 30 mM 嘉磷塞，但其機制未被證明(Forlani *et al*. 1992)。菜豆(*Phaseolus vulgaris* L.)不同 5 品系經藥劑篩選出的耐性株，EPSPS 基因發生單點變異，於第 437 的 glutamic acid(E)胺基酸改變為 glutamine(Q)(Xiang *et al.* 2001)。

於無嘉磷塞的培養環境下，不同植物細胞株之耐性穩定度亦不同。可保有耐性者包括菊苣、番茄及菸草(Smith *et al*. 1986; Goldsbrough *et al*. 1990; Sellin *et al*. 1992)，有些植物對嘉磷塞的耐性會減少或喪失，長春花於 6 個月後喪失 15%耐受性(*Cresswell* et al. 1988)，野生胡蘿蔔於 2 年後喪失一半耐性(Murata *et al*. 1998)。甜玉米 Black mexican 品系細胞的耐性，不具可遺傳性(Forlani *et al*. 1992)。菸草耐性細胞株發育為植株之後，耐受性皆消失(Singer and McDaniel 1985)；但是 Dyer 等(1988)研究顯示菸草的耐性可維持於再生植物。此外，嘉磷塞會造成番茄耐性細胞株生長的延遲(Nafziger *et al*. 1984)。經由細胞篩選而發展出來的耐嘉磷塞植物，其植株內缺乏可遺傳的抗性特質，只有於嘉磷塞長時期存在時，才會發生基因增幅的耐性機制(Papanikou et al. 2004)，因此以藥劑篩選抗嘉磷塞細胞株的途徑，並不適於發展為商品化的抗性作物。

抗嘉磷塞之低敏感度 EPSPS

一、胺基酸置換形成之嘉磷塞抗性

嘉磷塞抑制 shikimic acid 路徑中 EPSPS 的作用，經由估算 appKi〔glyphosate〕數值，可知 EPSPS 與嘉磷塞的親和力，Ki 值愈高表示抗性愈強，由 appKm〔PEP〕亦可估算 EPSPS 與受質 PEP 鍵結的親和力，Km 愈低表示 EPSPS 愈易與 PEP 鍵結而產生 EPSP 產物，若 EPSPS 基因發生變異，造成 Ki 值增加，則可增強對嘉磷塞的抗性，但若同時亦造成 Km 值的增加，則不利於 shikimic acid 路徑的作用。表二為 5 種微生物及 2 種植物 EPSPS 點突變後，其 EPSPS 活性、Ki 及 Km 值之比較。

土壤細菌(*Salmonella typhimurium*)篩選出之菌株，其 aroA 基因具有抗嘉磷塞的特性(Comai *et al*. 1985)，其中於第 101 個胺基酸 proline(P)變異為

serine(S)，EPSPS 酵素的比活性為敏感者的 2 倍(Stalker *et al*. 1985)。將此 aroA 基因轉殖於菸草及番茄，再生植株皆具抗性(Comai *et al*. 1985; Fillatti *et al*. 1987)。*S. typhimurium* M12 菌株的 aroA 基因於 35、40、42、83、185、201 及 230 處，共置換了 7 個胺基酸，Ki 值增加 19 倍，Km 值減少 23 倍(He *et al*. 2002)。類似單一胺基酸改變的大腸桿菌(*Escherichia coli*)及 *Klebsiella pneumoniae* (Kishore *et al*. 1986, 1987; Sost and Amrhein 1990)，二者皆於第 96 個胺基酸 glycine(G)改變為 alanine(A)，*K. pneumoniae* 突變株及正常株的 Ki 值分別為 8 mM 及 1 μM，大腸桿菌突變株及正常株的 Ki 值則分別為 4.1 mM 及 0.5 mM，明顯降低對嘉磷塞的親和力，增強抗性。若將 *S. Typhimurium* 及 *E. coli* 第 42 個胺基酸 threonine(T)改變為 methionine(M)，則 Ki 值增加 20-26 倍，且 Km 值降低 9-25 倍，EPSPS 的比活性分別增加 4.7 及 23.5 倍(He *et al*. 2003)。

矮牽牛的 *EPSPS* 基因利用點突變方式，改變第 101 個胺基酸 glycine(G)為 alanine(A)，突變株及正常株的 Ki 值分別為 2.0 mM 及 0.4 μM，Ki 值增加 5,000 倍，Km 值亦增加 42 倍(Padgette *et al*. 1991)，此矮牽牛變異株尚無法抗嘉磷塞田間施用劑量(Bradshaw *et al*. 1997)。經由第 2 個胺基酸改變的雙突變處理，包括第 144、167 或 192 個胺基酸分別變更為 aspartic acid(D)、serine(S)或 threonine(T)，Km 值皆比僅第 101 處變異者低，但 Ki 值亦相對的降低(Padgette *et al*. 1996)，此雙突變經轉殖後只略增加抗性，仍不具商品化應用性。自然突變牛筋草(*Eleusine indica* L.)的第 106 個 proline(P)改變為 serine(S)，酵素活性為感性株的 5 倍，Ki 及 Km 值分別增加 16 倍及減少 2 倍(Baerson *et al*. 2002, Powles and Preston 2006)。市售基改玉米之 GA21 品系為雙突變點轉植株，以人為突變修飾第 102 個 threonine(T)及第 106 個 proline(P)胺基酸，分別改變為 Isoleucine(I)及 serine(S)，其中第 106 胺基酸的改變與牛筋草(*Eleusine indica* L.)者相同，轉基因的構築包括水稻(*Oryza sativa* L.) actin 基因啟動子、向日葵(*Helianthus annuus* L.)及玉米 ribulose-1,5-bisphosphate carboxylase oxygenase small subunit (RuBisCo SSU)基因的葉綠體訊息胜肽(chloroplast transit peptide, CTP)、突變的玉米 EPSPS 基因及 nos 終結子(terminator)(Fig. 1A)(Yuan *et al*. 2006; GM Database from AGBIOS, 2007)，GA21 基改玉米的 EPSPS 只修飾 2 處胺基酸，即可有效抗嘉磷塞的田間施用劑量 (Gower *et al*. 2002, 2003; Lebrun 1997; Tharp and Kells 1999)。

二、抗嘉磷塞之 CP4 EPSPS

商品化抗嘉磷塞轉基因作物，包括大豆、棉花、玉米、甜菜及油菜等，

其轉基因大部份由農桿菌(*Agrobacterium* sp.) strain CP4 中選殖出之 CP4 EPSPS(Barry *et al*. 1992; Padgette *et al*. 1991)。CP4 EPSPS 為目前對嘉磷塞最具抗性的酵素，其 Ki 及 Km 值分別為 2.7 mM 及 12 μM，轉基因的啟動子多數為修飾過的花椰菜嵌紋病毒啟動子(CaMV 35S promoter)，抗性作物 CP4 EPSPS 轉基因可大量表現於植體中，如玉米 NK603 品系及大豆 GTS 40-3-2 品系的抗性基因為 CP4 EPSPS，但轉基因的構築組成不同，玉米 NK603 品系，含 2 組轉基因，主要差別於 35S 及 actin 啟動子(Fig. 1B)；大豆 GTS 40-3-2 品系含 1 組轉基因，包括 35S 啟動子、矮牽牛葉綠體訊息胜肽、CP4 EPSPS 及 nos 終結子(Fig. 1C) (Yuan and Chiang 2002, 2006; GM Database from AGBIOS, 2007)。大部份抗嘉磷塞基改作物的產量與非基改者無顯著差異(Delannay *et al*. 1995; Elmore *et al*. 2001)，但抗嘉磷塞棉花於幼齡植株噴施嘉磷塞，其繁殖器官對嘉磷塞忍受性降低現象，數月後植體中仍有藥劑殘留(Jones and Snipes 1999; Pline *et al*. 2002)。嘉磷塞的長效或是 CP4 EPSPS 無法有效表現時，可能造成敏感部位的傷害 (Pline *et al*. 2002)，發展分解嘉磷塞為無毒代謝物的抗性機制，是未來抗性策略的較佳選擇。

代謝嘉磷塞之機制

嘉磷塞可被多種微生物分解代謝(Torstensson and Aamisepp 1977; Moore *et al*. 1983)。土壤微生物的代謝方式有二：形成 sarcosine 中間產物者，稱為 C-P lyase 路徑；將嘉磷塞代謝為 aminomethyl phosphonic acid (AMPA)及 glyoxylate 者，稱為 AMPA 路徑(Ruff *et al*. 1991; Torstensson 1985)。嘉磷塞於植物體內亦可被代謝(Duke *et al*. 2003; Komossa *et al*. 1992)，大豆可將嘉磷塞代謝為 AMPA， Franz *et al*. (1997)推測可能是葉片表面的微生物將嘉磷塞代謝為 AMPA 而測得之故，並非大豆本身的代謝作用，Duke 等(2003)於嘉磷塞處理的大豆種子，測得 AMPA，顯示大豆可能有代謝嘉磷塞的未知酵素。

首度應用於代謝嘉磷塞的轉基因，由土壤細菌(*Ochronobactrum anthropi*) LBAA strain 選殖之 glyphosate oxidoreductase(*GOX*)基因，其編碼的 GOX 酵素可分解嘉磷塞 C-N 鍵，形成 AMPA 及 glyoxylate(Fig. 2A) (Barry *et al*. 1992)。將 *GOX* 基因修飾後轉殖於菸草，可產生耐嘉磷塞的轉基因菸草，另外應用於油菜及其他作物亦具有抗嘉磷塞田間施用劑量的效果(Franz *et al*. 1997)。美國登記上市的抗嘉磷塞甜菜、小麥、玉米及油菜，皆已發展含有 *GOX* 及 CP4 *EPSPS* 基因的品系，如玉米 MON832 品系(Fig. 1D)、油菜 GT200 品系 (Nap *et al*. 2003; Zhou *et al*. 1995)，抗嘉磷塞棉花則只有轉殖 CP4 *EPSPS*

的品系(Nida *et al*. 1996)。然而嘉磷塞的 AMPA 中間產物對轉基因大豆具有輕微毒性(Hoagland 1980; Reddy *et al*. 2004)，其他含 *GOX* 基因的轉殖作物則尚無相關的毒性報導。

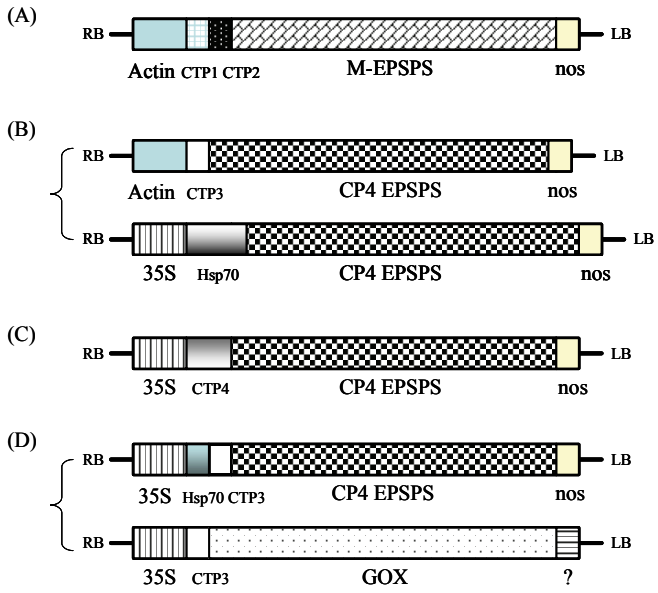

Fig. 1. Introduced genetic elements of glyphosate-resistant crops. (A) maize GA21, (B) maize NK603, (C) soybean GTS 40-3-2, (D) maize MON832. 35S, cauliflower mosaic virus promoter; actin, actin I promoter and intron sequences from rice; NOS, nopaline synthase ternimator; CP4 EPSPS, 5-enolpyruvyl shikimate -3-phosphate synthase (EPSPS) gene from *Agrobacterium* sp. strain CP4; M-EPSPS, modified EPSPS gene from corn, GOX, glyphosate oxidoreductase gene from *Ochronobactrum anthropi*; CTP1, chloroplast transit peptide of ribulose-1,5-bisphosphate carboxylase oxygenase small subunit (RuBisCo SSU) gene from sunflower; CTP2, chloroplast transit peptide of RuBisCo SSU gene from corn; CTP3, chloroplast transit peptide of RuBisCo SSU gene from Arabidopsis; CTP4, chloroplast transit peptide from petunia; HSP70, heat shock protein 70 from maize.

另一代謝嘉磷塞的酵素蛋白基因，由地衣芽孢桿菌(*Bacillus licheniformis*)選殖之 glyphosate N-acetyl transferase (*gat*)基因，其編碼的 GAT 酵素可將嘉磷

塞經由 N-acetylation 代謝(Fig. 2B) (Castle *et al*. 2002, 2004; Siehl *et al*. 2005)，此作用機制與抗固殺草(glufosinate)基改作物者相似，*bar* 或 *pat* 基因編碼的

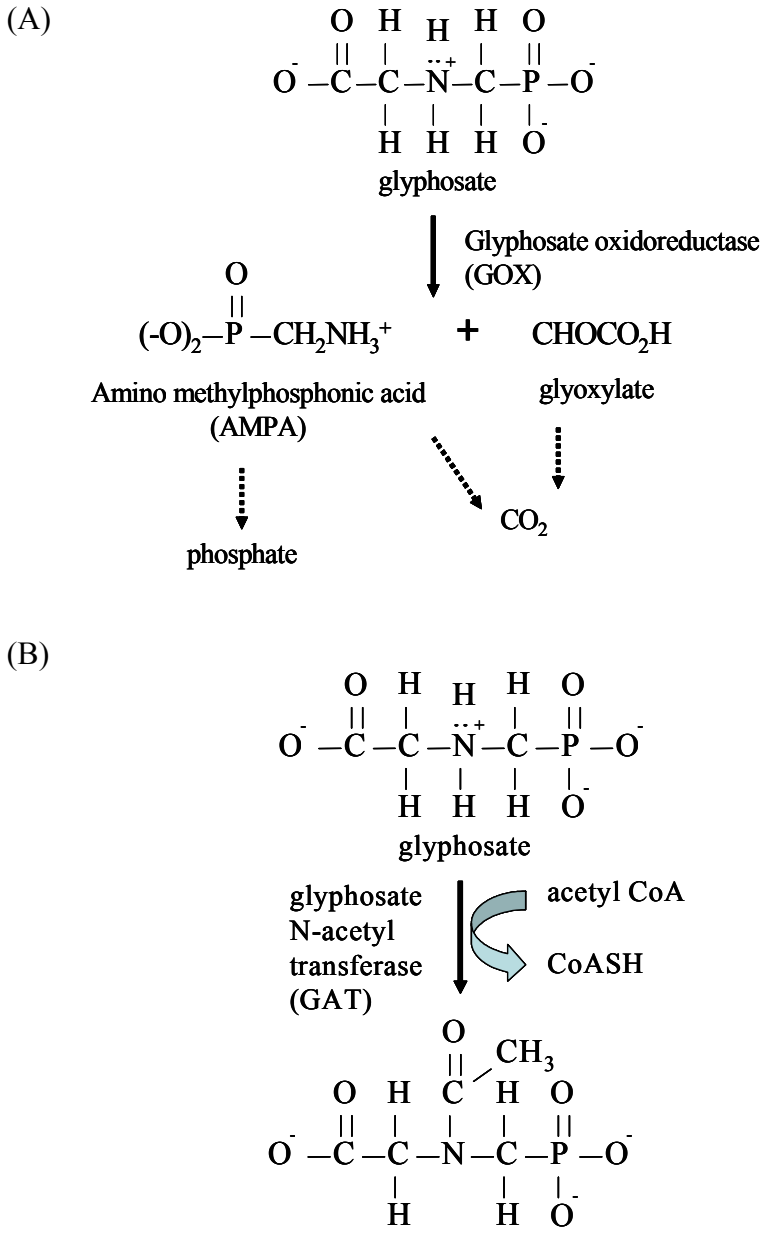

Fig. 2. The glyphosate oxidoreductase (A) and acetyltransferase(B) reaction. (Barry *et al*. 1992; Siehl *et al*. 2005; Zhou *et al*. 1995)

phosphinothricin N-acetyl transferase(PAT)酵素，亦催化 N-acetylation 作用而代謝固殺草(Dröge *et al*. 1992; Thompson *et al*. 1987)，含 *gat* 基因的轉殖作物尚處於研究階段，目前沒有通過登記的品系。

結　論

抗嘉磷塞作物可依雜草生長狀態噴施嘉磷塞，取代萌前藥劑使單位面積施藥量減少，同時可實施不整地栽培，大幅降低生產成本，因此全球抗嘉磷塞作物的種植面積持續增加。抗嘉磷塞作物的抗性機制有三種：包括產生大量 EPSPS 酵素、轉殖對嘉磷塞低親和性的 EPSPS 基因，以及轉殖代謝嘉磷塞的基因。此三種抗性策略以選用代謝解毒之機制，可以避免基改作物的藥劑傷害，同時降低藥劑於植物體內的殘留，優於對藥劑低親和性基因的利用。由於抗嘉磷塞基改作物持續大面積使用嘉磷塞，導致多種抗嘉磷塞雜草出現，如禾本科的牛筋草、菊科的加拿大蓬(*Conyza canadensis* (L.) Cronq.)、美洲假蓬(*C. bonariensis* (Linn.) Cronq.)，以及莧科的 *Amaranthus rudis* Sauer 及 *A. palmeri* S. Watson 等，雜草抗性的機制除了牛筋草 EPSPS 的點突變之外，加拿大蓬的抗性可能涉及嘉磷塞於植體內的轉運不良，或是多種機制共同的結果，其他多數雜草的抗性原因仍不明確。雜草抗藥性管理及利用，是未來發展抗嘉磷塞基改作物需克服及解決的問題。

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