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Beyond sweetness: Rethinking the use and conservation of *Stevia rebaudiana*'s plant genetic resources for breeding

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Abstract

This work analyses the genetic potential of *Stevia rebaudiana* beyond its sweetening properties, aiming to reassess its conservation and utilization in breeding programmes. This focus is justified by the growing global demand for natural sweeteners and the challenges posed by extreme weather events and diseases affecting crop yield and quality. The relationship between the species' genetic diversity and its response to biotic and abiotic stresses is explored, as well as the limitations of current conservation and breeding strategies. Findings reveal underutilized genetic resources, limited integration of genomic tools in breeding efforts and a lack of robust *in situ* conservation initiatives. Genotypic variability has been observed in response to diseases such as Septoria leaf spot, while abiotic factors, including salinity and drought, can significantly influence steviol glycoside content. It is concluded that an integrated approach, combining dynamic conservation, photoperiod-informed breeding strategies, improvements in seed viability, omics-based tools and coherent public policies, is essential to ensure crop resilience and genetic sovereignty, especially in regions of origin such as Paraguay. The sustainable use of *Stevia*'s genetic diversity could foster more sustainable, healthier and fairer production systems.

Introduction

Growing interest in natural sweeteners as sugar alternatives has driven global demand for stevia (*Stevia rebaudiana* [Bertoni] Bertoni). Its steviol glycosides, which offer greater sweetness and no caloric impact, constitute a promising product amidst the rise of metabolic diseases such as obesity and diabetes. However, *Stevia rebaudiana*'s potential as a leading source of non-caloric sweeteners is not limited solely to its concentration of sweet compounds but also lies in the need for genetic improvement. There is still underutilized genetic diversity, which is essential for improving sensory quality, yield gain, generation of extreme weather-resilient cultivars and increased resistance to diseases that jeopardize production (Mereles *et al.*, 2013).

The wild relative species of stevia exhibit significant intraspecific genetic variability, creating opportunities for breeding programmes aiming to improve agronomic traits, biochemical profiles and adaptability to varied environmental conditions (Yadav *et al.*, 2011; Gantait *et al.*, 2018). The combined exploitation of plant genetic resources and wild relatives is central to the development of improved cultivars that can overcome limitations such as unstable glycoside composition, and strong sensitivity to photoperiod (Abdullateef and Osman, 2011). Over the past decades, characterizations based on morphological, agronomic, biochemical and molecular studies have revealed a broad spectrum of genetic materials available for selection and genetic enhancement (Chester *et al.*, 2013).

Despite considerable progress in characterizing both cultivated and wild stevia germplasm, key questions remain about how to integrate underutilized diversity into breeding pipelines for enhanced sweetness, yield stability and stress resilience. This review, therefore, synthesizes the current state of genetic-resource knowledge across *Stevia rebaudiana* and its wild relatives, and proposes an integrated framework combining omics, genome editing, genetic studies and molecular breeding to accelerate the development of environment-adapted and pathogen-resistant cultivars.

Wild relatives of *Stevia rebaudiana*

The genus *Stevia* includes approximately 230 species, among which *Stevia rebaudiana* is notable for accumulating sweet steviol glycosides (Borgo *et al.*, 2021). Wild relatives, such as

S. eupatoria, S. lemmonii and *S. micrantha*, occur from the southern United States to Argentina, occupying diverse ecological niches and exhibiting cytogenetic variability (diploid, triploid, tetraploid) that underlies a multibasic genetic structure (Yadav *et al.*, 2011; Borgo *et al.*, 2021). Although not all wild species produce high glycoside levels, they possess adaptive traits, morphological differences in leaf architecture and growth habit, plus mechanisms to tolerate drought, fluctuating light and temperature extremes, that can be introduced into cultivated lines via conventional or biotechnological breeding to enhance biomass and glycoside yield under suboptimal conditions (Brandle *et al.*, 1998; Borgo *et al.*, 2021; Gantait *et al.*, 2018; Al-Taweel *et al.*, 2021).

Because landraces and wild types often display greater genetic diversity and a higher number of private alleles than bred cultivars, these relatives serve as reservoirs of alleles for disease resistance, yield stability and steviol glycoside biosynthesis (Cosson *et al.*, 2019; Borgo *et al.*, 2021; Gantait *et al.*, 2018). Molecular and cytogenetic analyses confirm that introgressing wild alleles can broaden the genetic base of commercial stevia cultivars, helping to overcome narrow genetic bottlenecks and improve overall performance (Yadav *et al.*, 2011; Gantait *et al.*, 2018).

Intraspecific variability and cultivar diversity within *Stevia* genus

Stevia rebaudiana is a perennial shrub 60–120 cm tall, with opposite oval leaves with serrated margins, leathery texture, green stems, white-flowered panicle inflorescences, taproots and a fragrant sweet herbal aroma (Fig. 1). This species exhibits extensive varietal diversity, comprising over 90 varieties adapted to various climatic requirements and production systems (Angelini *et al.*, 2018). Many studies have demonstrated that significant heritability exists for key yield components, such as leaf yield and glycoside concentrations, indicating that genetic improvement is feasible through recurrent selection and breeding (Yadav *et al.*, 2011). Modern germplasm collections incorporate both landraces and improved clones that have been developed through selective breeding and vegetative propagation methods (Clemente *et al.*, 2021). Evaluations of genetic variability using molecular markers, such as Random Amplified Polymorphic DNA (RAPD), Expressed Sequence Tag – Simple Sequence Repeat (EST-SSR) and Single Nucleotide Polymorphism analyses, have confirmed the presence of valuable polymorphisms underlying differences in key traits such as glycoside composition and stress tolerance (Cosson *et al.*, 2019). Detailed biochemical and molecular profiling of these materials reveals a wide range of steviol glycoside concentrations and complementarity in metabolic pathways, making them desirable targets for further improvement (Hastoy, 2018).

Moreover, in trials of four elite stevia populations in North Carolina, key growth traits proved to be highly heritable, suggesting they respond well to selection (Kozik *et al.*, 2020). For example, plant height exhibited broad-sense heritability values of 0.68 in June and 0.60 in August, branch width showed heritabilities of 0.59 and 0.55, and leaf area came in at 0.54 and 0.52. Under a 20% selection intensity, breeders could expect annual gains of roughly 1.2 Mg ha^{-1} in dry biomass and about 24 mm in height. Similarly, the major steviol glycosides displayed strong genetic control: rebaudioside A had $H^2g = 0.60$, rebaudioside C was 0.58, rebaudioside D was 0.50, stevioside was 0.52 and total steviol glycosides (TSGs) reached 0.62. These values translate to potential per-cycle increases of about 26.4 mg g^{-1} for rebaudioside A (14.5 % of TSG) and 20.2 mg g^{-1} for rebaudioside C (20.2% of TSG). Given this level of genetic variation, adopting marker-assisted selection makes it possible to accelerate improvements in both yield and glycoside composition.

Sexual reproduction as a bottleneck in the commercial scale-up of *Stevia rebaudiana*

Stevia rebaudiana exhibits two modes of reproduction: sexual propagation via seeds and asexual propagation via stem cuttings or tissue culture, each contributing differently to genetic diversity and uniformity in commercial plantations (Ramakrishnan *et al.*, 2025). However, seeds present several challenges that limit their utility. *Stevia rebaudiana* individual seeds develop within achene-type fruits that act as the primary propagule or dispersal unit. Dark-coloured achenes tend to be heavier due to successful fertilization and embryo formation, yet they show highly variable viability, with germination rates ranging from less than 1% to as high as 59–86%. This variability in seed germinability results from intrinsic issues with pollination and fertilization compatibility, often leading to malformed embryos or endosperm. Consequently, viable seeds exhibit a wide range of physiological vigour, affecting key germinability parameters such as germination rate, uniformity and speed within a single seed batch – ultimately impairing seedling stand uniformity and crop establishment (Joosen *et al.*, 2010).

In contrast, light and tan achenes are typically lighter because they result from self-incompatibility processes that inhibit viable seed formation, producing empty achenes or seeds without embryos, which fail to germinate. Furthermore, a sporophytic self-incompatibility mechanism renders most self-pollinated seeds infertile, requiring cross-pollination to produce seeds. This results in genetically heterogeneous half-sib progenies and inconsistent steviol glycoside profiles (Angelini *et al.*, 2018).

Additional reproductive and environmental factors, including premature seed harvest, protandry, low pollen viability, nutrient deficiencies affecting pollen tube growth and the need for specific light and temperature conditions, further depress viable seed production (Al-Taweel *et al.*, 2021). Moreover, stevia seeds are extremely small ($\sim$3 mm), contain minimal endosperm reserves and deteriorate rapidly unless stored at low temperatures. These characteristics hinder seed handling, storage and quality maintenance, making sexual propagation inefficient for large-scale multiplication due to poor, inconsistent germination rates and genetically mixed offspring (Sharma *et al.*, 2023).

Consequently, vegetative propagation, either via stem cuttings or *in vitro* micropropagation, has become the preferred method for ensuring genetic uniformity, achieving higher propagation success, and faster multiplication of selected clones with consistent phytochemical profiles (Abdullateef and Osman, 2011; Al-Taweel *et al.*, 2021; Khan *et al.*, 2021).

Linking genetic resources with strategies to cope with biotic and abiotic stress in stevia cultivation

Although less documented than in other crops, several biotic stressors, such as foliar and soilborne pathogens, significantly reduce yield and quality of stevia. Notable examples include Septoria leaf spot caused by *Septoria steviae* (Sanabria-Velazquez *et al.*, 2024), foliar spots due to *Alternaria steviae* and *A. alternata* infections (Maiti *et al.*, 2007; Yan *et al.*, 2018), and rots caused by *Sclerotinia sclerotiorum* and *Rhizoctonia solani* (Koehler *et al.*, 2019; Kessler and Koehler, 2020). Likewise, *Fusarium oxysporum* causes vascular wilt (Díaz-Gutiérrez *et al.*, 2019), and other secondary pathogens

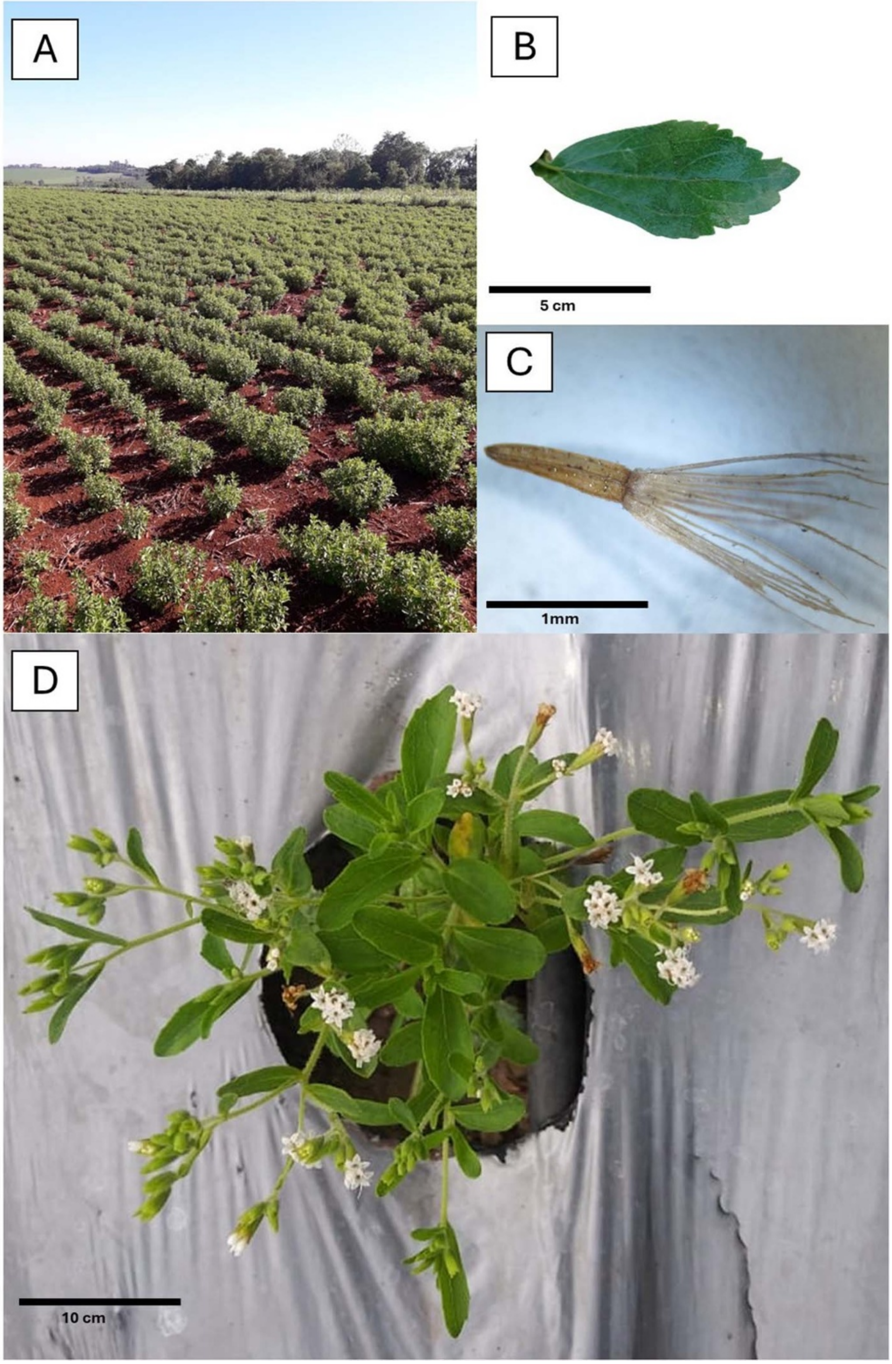

Figure 1. Morphological features and cultivation of *Stevia rebaudiana*. (A) Field cultivation of stevia plants in a commercial production area in the South of Paraguay. (B) Stevia leaf showing its characteristic serrated margin and elongated oval shape (scale bar = 5 cm). (C) Stevia seed (achene) with its pappus hairs aiding in wind dispersal (scale bar = 1 mm). (D) Flowering stevia plant grown under plastic mulch conditions, displaying inflorescences with small white flowers and opposite leaves (scale bar = 10 cm).

such as oomycetes, bacteria, viruses, phytoplasmas and various fungi may also be present (Samad *et al.*, 2011; Chatzivassiliou *et al.*, 2015; Koehler and Shew, 2017; Rogers and Koehler, 2021; Sanabria-Velazquez *et al.*, 2023). Currently, studies reporting the extent to which the above-mentioned pathogens affect stevioside content are scarce. However, infections caused by *A. alternata* have been observed to reduce the commercial value and quality of stevia leaves, resulting in a negative impact on stevioside concentration (Prakash *et al.*, 2022), although no specific quantitative data have been published. Since leaf quality is essential for stevioside content, it is reasonable to consider that any disease compromising leaf integrity could decrease the concentration of these compounds (Arturo *et al.*, 2009). Nonetheless, further studies are needed to quantify these effects and better understand the relationship between infections by these pathogens and stevioside content in stevia.

Additionally, abiotic stresses, including drought, salinity, waterlogging and chemical treatments, can significantly alter steviol glycoside accumulation and plant growth. For instance, salinity and drought stress have been found to enhance the concentrations of stevioside and rebaudioside A, although they simultaneously limit biomass accumulation (Debnath *et al.*, 2019). Moreover, Gupta *et al.* (2016) highlighted that stevia plant treated with compounds of diverse nature such as Na_2CO_3, proline and polyethylene glycol, displayed specific metabolic changes, which in some cases enhanced TSG production by up to threefold compared to non-treated plants, despite negatively impacting shoot growth. The fact that the expression of several members of the *SrMYB* transcription factor family which is one of the largest regulators of gene expression and hence a variety of plant functions, is tissue-specific, stress responsive and highly associated with steviosides content, turn them into potential targets for engineering weather extreme resilient varieties and enhanced stevioside profile (Chen *et al.*, 2024). Therefore, it is necessary to elucidate the molecular basis that regulates stevia physiological responses to stress combination, presence of both biotic and abiotic stressors, to ensure yield stability under adverse climatic conditions.

The species' ability to withstand or tolerate these biotic and abiotic stressors largely depends on the genetic variability present in its populations. Several studies suggest that, while no genotype shows complete resistance to major pathogens, there is significant variation in how different lines respond to infections like Septoria leaf spot. Hastoy *et al.* (2019) have evaluated the response to *Septoria* sp. of 10 genotypes from different origins and identified 2 of them, 'Gawi' and 'Esplac1', as moderately susceptible, with only 10–15% of symptomatic leaf area, whereas 3 highly susceptible genotypes, named 'E8', 'C' and 'E161718', which reached up to 40% of symptomatic leaf area, were also reported. In a leaf-disk assay, Le Bihan *et al.* (2025) have reported minimal severity (3% symptomatic area) for 'Cult102_SPA' and 'Cult76_GER' genotypes confirming their lower susceptibility. Moreover, Yadav *et al.* (2011) highlighted the line SF5-1 (No. 103) for its enhanced resistance against *Septoria* sp., and Huber and Wehner (2021) have found significant variability in disease resistance, biomass yield and glycoside content across various seed-derived cultigens, which emphasizes the pivotal role of genetic studies in unravelling the molecular basis underlying resistance to pathogen attack in *stevia* genotypes, while highlighting the importance of conserving and utilizing plant genetic resources for breeding programmes aimed at developing genotypes with superior adaptive profiles (Ramakrishnan *et al.*, 2025).

Regarding cold stress, a study conducted by Kozik *et al.* (2020) exposed 14 different stevia lines to cold temperatures for up to 10 days. They discovered that only one line, 7947-3, showed almost no damage after six days at −2°C. When the temperature was held at 0°C for 8 days, a small handful of lines (including 7947-3 and 7990-17) still looked healthy, while the others began to suffer. Even at a milder 2°C, it took 10 days before most plants showed signs of distress, yet lines 7947-3, 7918-1 and 7686-6 continued to hold up well. Based on these results, a simple way to screen for cold tolerance is to check leaf damage after 6 days at −2°C, 8 days at 0°C, or 10 days at 2°C, all under a light intensity of about 500 μmol m^{-2} s^{-1}. Therefore, lines like 7947-3, which stay largely unscathed after these treatments, are especially valuable because they offer the potential to develop stevia varieties capable of thriving in temperate regions.

Improving steviol glycoside profile

A glycoside contains glucose and non-sugar parts derivate from the diterpenoid steviol, and in stevia the major ones are named stevioside and rebaudioside A (Reb A) (Humphrey *et al.*, 2006; Brandle and Telmer, 2007; Zhou *et al.*, 2021). These compounds share a common precursor (kaurenoid) with that of the plant hormone gibberellic acid and their production occurs mainly in leaves. The first step of steviol glycoside biosynthesis pathway and its related genes is very well described (Bondarev *et al.*, 2003). In brief, through the action of four consecutive enzymes, geranylgeranyl-diphosphate is converted into steviol, followed by its glycosylation, via specific glycosyltransferases to form different steviol glycosides (Sharma *et al.*, 2023). Several studies have reported attempts to improve steviol glycosides through genetic transformation and metabolic pathway engineering. For instance, plants overexpressing *UDP-glycosyltransferase 76G1*(*SrUGT76G1*), enzyme that catalyses stevioside to Reb A conversion, displayed a significant increase of Reb A: stevioside ratio, which also improved organoleptic properties (Richman *et al.*, 2005; Kim *et al.*, 2019). Note that 68 putative *UGTs* have been identified in stevia, and particularly, the functionality of *SrUGT76G1* was characterized through transient expression in *Nicotiana benthamiana*. This approach could be used to study the genetic control of steviol glycoside composition in this crop, which remains unknown (Petit *et al.*, 2020). Concordantly, Zheng *et al.* (2019) reported that transgenic plants overexpressing 1-deoxy-*d-xylulose-5-phosphate synthase 1* (*SrDXS1*) and *kaurenoic acid hydroxylase* (*SrKAH*), both enzymes involved in steviol synthesis, showed enhanced content of steviol glycosides, up to 42–54% and 67–88%, respectively.

In this context, Bogado-Villalba *et al.* (2021) assessed the genetic relationship among several Paraguayan *Stevia rebaudiana* lines and varieties, along with their steviol glycoside profile using SSR and ISSR markers. Genotyping revealed two main clusters, one of which was predominantly composed of Eriete and Katupyry varieties, as well as other lines characterized by high steviol glycoside content. Similar clustering pattern have been reported by Bhandawat *et al.* (2014), Dyduch-Siemińska *et al.* (2020) and Subositi *et al.* (2011), with Cosson *et al.* (2019) identifying three clusters across 145 global genotypes using EST-SSR markers. These studies collectively demonstrate how molecular markers can unravel genetic structure and diversity, facilitating the identification and incorporation of valuable traits into stevia breeding programmes (Barbet-Massin *et al.*, 2016).

Photoperiod plasticity and polyploidy as breeding targets for enhancing biomass and environmental resilience

Stevia rebaudiana is an obligate short-day plant with a critical day length of 13 h (Ramesh *et al.*, 2006), making its phenology highly sensitive to day length. Interestingly, several studies have reported enhanced stevia production under long-day conditions, likely due to reduced glycoside synthesis at or near flowering. Under extended photoperiods, genes for steviol glycoside biosynthesis are upregulated in leaves, while gibberellin-related genes are downregulated; in stems, gibberellins increase, promoting elongation and biomass (Yoneda *et al.*, 2017; Gantait *et al.*, 2018; Rengasamy *et al.*, 2022; de Andrade Mv *et al.*, 2023). These responses can inform crop strategies, including artificial lighting or breeding, to boost vegetative growth and glycoside content. Photoperiodic responses in *Stevia rebaudiana* indicate the need for distinct cropping protocols: short days favour flowering and seed production, while long days or extended lighting enhance vegetative growth and glycoside yield. Optimizing protocols for each purpose, vegetative production or seed harvesting, requires aligning cultivation with suitable latitudes or seasons to meet photoperiod needs and maximize crop performance.

Stevia rebaudiana is cultivated across Asia, South America and North America, where it encounters diverse environmental conditions, including varying day lengths. Despite this wide distribution, no stevia species have shown long-day photoperiodic behaviour.

A comprehensive phenological and physiological assessment of accessions from different latitudes is essential to uncover naturally occurring allelic diversity and to optimize cultivation under contrasting photoperiods (de Oliveira Vm *et al.*, 2004; Ramesh *et al.*, 2006; González-Delgado *et al.*, 2025; Ramakrishnan *et al.*, 2025).

Genome-wide association studies (GWAS) are powerful tools to explore natural genetic variation in wild stevia and identify loci linked to adaptive traits like photoperiodic responses. Applying GWAS to native accessions may reveal quantitative trait loci (QTLs) associated with day-length sensitivity, improving understanding of ecological adaptation and supporting breeding for environmental suitability. Additionally, identifying stevia orthologs of *florigen* and components of the *Arabidopsis* circadian clock could enable targeted genetic engineering. This foundational knowledge may facilitate manipulation of growth habit genes, supporting the development of cultivars with extended vegetative phases, indeterminate growth or reduced photoperiod sensitivity – traits successfully modified in crops like tomato and sorghum (Murphy *et al.*, 2011, 2014; Klein *et al.*, 2015; Vicente *et al.*, 2015).

Polyploidization, the presence of three or more chromosome sets, can be induced in stevia using colchicine and has been applied as a breeding tool. Autotetraploid plants often develop larger and thicker leaves than diploids, increasing total glycoside yield (Yadav *et al.*, 2013; Xiang *et al.*, 2019; Joshi *et al.*, 2025). Beyond enhancing plant and organ size, polyploidy plays a key role in stress responses (Bhosale *et al.*, 2019; Lang and Schnittger, 2020). Stress conditions such as heat and drought have been shown to raise ploidy levels, indicating a general adaptation mechanism involving upregulation of metabolic and defense-related genes (Cookson *et al.*, 2006; Monjardino *et al.*, 2006; Scholes and Paige, 2015; Tossi *et al.*, 2022). In support, Markosyan *et al.* (2021) found that polyploidy modified resistance gene expression in stevia, while in *Arabidopsis*, increased ploidy correlated with changes in cell wall structure linked to enhanced pathogen resistance (Hamdoun *et al.*, 2016; Bhosale *et al.*, 2018).

The genus stevia exhibits considerable chromosomal variation, with basic numbers of $x = 11$, 12 or 17. Diploid ($2n = 22$), triploid ($2n = 33$) and tetraploid ($2n = 44$) species have been documented (Yadav *et al.*, 2011). Polyploid populations often differ ecologically and morphologically from diploids (de Oliveira Vm *et al.*, 2004). In Mexican stevia species, agamospermous polyploids occupy broader geographic ranges than diploids, suggesting greater colonization potential and ecological adaptability (Watanabe *et al.*, 2001). Their dominance in marginal habitats highlights polyploidy's role in adaptation and range expansion. This natural chromosomal variation offers valuable genetic resources for breeding, especially to enhance resilience to environmental stress. Polyploid individuals, particularly those reproducing agamospermously, tend to exhibit reproductive stability and greater abiotic stress tolerance. Selecting triploid and tetraploid genotypes from diverse habitats could allow breeders to capture beneficial ploidy-related traits, such as increased leaf biomass and stress tolerance, without requiring artificial chromosome doubling.

Genome editing as a tool to harness the natural variation of stevia wild relatives

Although breeding efforts face methodological and conceptual challenges, genotypes with more desirable glycoside profiles have been identified. Nonetheless, a systematic framework for generating pathogen-resistant and enhanced glycoside profile varieties with large-scale availability still does not exist. The genetic complexity underlying steviol glycoside synthesis, combined with fragmented information on key genes, metabolic pathways and factors of disease resistance, limits the rational design of superior varieties (Singh *et al.*, 2017; Xu *et al.*, 2021).

Biotechnological tools, including genetic editing, enable the molecular breeding of crops with specific properties. However, it requires an in-depth understanding of the genetics basis that controls development and physiology in the crop of interest (Younes *et al.*, 2019; Taak *et al.*, 2020; Biswas *et al.*, 2021). As previously described, this has been addressed for steviol glycoside biosynthesis and certain abiotic stress responses (Pal *et al.*, 2023). The combination of this fundamental biological knowledge and state-of-the-art gene editing techniques, such as CRISPR and TALEN, can be used to target a specific gene to improve traits in a precise manner. Another strategy that could be implemented in stevia breeding is called *de novo* domestication, which exploits the existence of wild relatives adapted to challenging environments and a wide range of photoperiod, traits with a diffuse polygenic basis, as a suitable raw material where monogenic domesticated-related and yield-determinant traits can be manipulated, instead of introducing alleles from wild relatives into cultivated crops. This approach is suitable for stevia breeding as plant transformation and delivery of CRISPR-based vectors have been successfully achieved in this crop and its genome is sequenced (Ghose *et al.*, 2022). Lastly, *de novo* domestication has been reported in tomato, maize and wheat (Zsögön *et al.*, 2017, 2018; Fernie and Yan, 2019).

Besides molecular tools, other approaches have been reported to be applied to enhance steviol glycoside levels. The variability observed in different *Stevia* genotypes in nature results from the accumulation of naturally occurring mutations, at a very low rate, throughout evolution. Hence, to produce novel genetic variation, mutations can be induced via physical and chemical mutagens (Raina *et al.*, 2016). Kumar *et al.* (2024) applied gamma ray to the stevia variety 'Madhuguna' to induce mutations, followed by selection of mutant with an improved steviol profile. This technique was found to be effective, at mild doses (5 and 10 kR), in improving steviol glycoside content.

Conservation and utilization of *Stevia* genetic resources: Bridging legal framework and breeding innovation

Conserving and harnessing *Stevia*'s genetic diversity is essential for creating lines with enhanced agronomic performance. Exploring this diversity enables researchers to identify genes that enhance disease resistance and stress tolerance, thereby reducing reliance on agrochemicals and promoting more sustainable production systems. Such variability also underpins stevia's ability to maintain stable yields amid extreme weather, as shifting weather patterns can influence pathogen prevalence and crop productivity.

Although market demand for stevia is growing, much of the available germplasm remains underused (Borgo *et al.*, 2021); therefore, it is crucial to evaluate how current conservation measures and breeding programmes preserve and deploy genetic variation, not only to refine sweetness and yield but also to bolster overall crop health and long-term resilience (Ramakrishnan *et al.*, 2025). It is crucial to continue characterizing existing germplasm banks. This ongoing effort allows us to accurately assess their current diversity and, critically, evaluate the need for an influx of new germplasm (Ribeiro *et al.*, 2021).

Conservation strategies have been predominantly limited to *ex situ* methods (seed banks, field collections) and *in vitro* propagation, lacking comprehensive plans that integrate wild diversity to

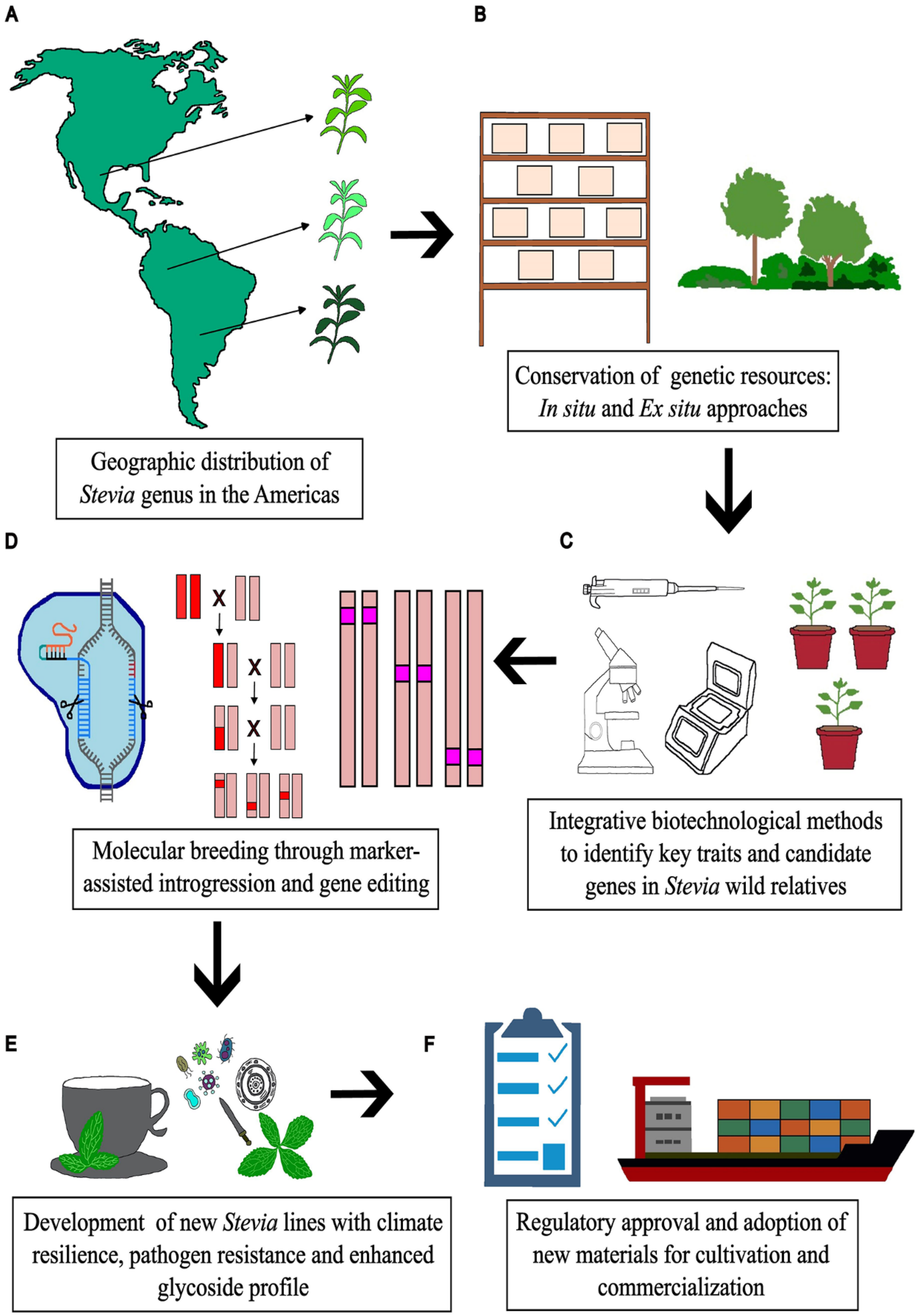

Figure 2. Proposed pipeline to promote conservation of *Stevia rebaudiana* genetic resources and their integration into breeding programmes and basic research. (A) The *Stevia* genus comprises close to 230 species, and their distribution areas extend from the southern United States to the South American Andean region. (B) This tremendous diversity should be conserved *in situ*, allowing the dynamic evolution of wild population and co-adaptation to local pathogens, or *ex situ*, under controlled environment where samples are stored (seedbanks, germplasms, gene banks, etc.). (C) Screening, through physiological and biochemical assays, of wild relatives to find key traits related to abiotic and biotic stress tolerance and steviol profile, followed by identification and selection of candidate genes or loci (QTL) via molecular tools. Altogether, via biotechnological approaches genes governing different economically important traits would be unravelled. (D) Introgression breeding and use of DNA marker technology in back cross programmes. Moreover, gene editing in breeding allows for precise manipulation of target traits by directly altering specific genes once their molecular basis has been identified. (E) Generation of new lines showing extreme weather resilient, pathogen attack resistance and improved glycosides profile. (F) Risk assessment and approval processes for the cultivation, consumption and commercialization of new materials. Lastly, adoption of the latter by growers and consumer markets.

meet producers/farmers and market needs. *In situ* conservation, which would safeguard the dynamic evolution of wild populations and their co-adaptation with local pathogens, has received scant attention (Salgotra and Chauhan, 2023).

Global conservation of genetic resources for crops like potato and maize has benefited from robust institutional support. The International Potato Center (CIP) in Peru maintains a comprehensive in-trust collection of virus-free potato germplasm using advanced cryopreservation protocols, ensuring safe and equitable distribution of genetic material worldwide (Vollmer *et al.*, 2017). Similarly, Brazil has developed a coordinated national system for germplasm conservation through Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), combining *in situ* and *ex situ* strategies including cryobanks for both plant and animal genetic resources, backed by international agreements and national laws protecting biodiversity and traditional knowledge (Machado *et al.*, 2016; Morrell and Mayer, 2017).

Moreover, international agricultural research centres such as Centro Internacional de Agricultura Tropical (CIAT) in Colombia have demonstrated the enormous economic value of the unrestricted flow of genetic resources across countries. For example, Latin American nations have significantly benefited from bean germplasm shared through CIAT, with improved varieties boosting agricultural productivity throughout the region, even in countries that did not originate the genetic material (Johnson *et al.*, 2005). This reinforces the argument that shared access to well-preserved and documented genetic resources can yield widespread and equitable benefits, especially when supported by transparent legal frameworks.

These initiatives required the support of public policies and legal frameworks that guarantee access, traceability and fair benefit-sharing. The case of *Stevia rebaudiana*, native to the Paraguay–Brazil border and traditionally used by the Guaraní people, illustrates the critical need for such mechanisms. Research has shown that major companies are using stevia-derived products as sugar alternatives in their products without consent from the Guaraní communities, raising serious concerns under the Convention on Biological Diversity and exposing legal gaps, particularly in Paraguay, which has not ratified the Nagoya Protocol. While Brazil has developed legal mechanisms for access and benefit-sharing, its laws exclude food products like stevia, further complicating Guaraní efforts to claim compensation or legal protection (Relly, 2023).

These examples show how the success of germplasm conservation and equitable access depends on institutional infrastructure, legal harmonization and long-term political will. To prevent future biopiracy and secure fair benefits for Indigenous peoples, especially in the case of stevia, it is urgent to consolidate similar international and national frameworks, such as those provided by CIP, CIAT or EMBRAPA, tailored to minor or neglected crops of high commercial interest.

New approaches and future perspectives

Integrating diversity from both cultivated germplasm and wild relatives remains a critical challenge in exploiting *Stevia rebaudiana*'s full genetic potential. The species' high heterozygosity and sporophytic self-incompatibility pose significant challenges to traditional breeding efforts, hindering the development of stable seed-based lines and requiring the use of precise molecular tools to assemble and fix desirable alleles (Yadav *et al.*, 2011; Ramakrishnan *et al.*, 2025). In this context, reproductive biology emerges as a fundamental yet often overlooked bottleneck that must be addressed through targeted research, especially given the increasing global demand for steviol glycosides and the need for scalable propagation systems.

The development of curated germplasm collections, with robust phenotypic and genotypic characterization, lays the groundwork for effective introgression strategies while minimizing linkage drag and preserving genetic integrity (Abdullateef and Osman, 2011; Hastoy, 2018). Wild relatives of stevia, adapted to environments from the Paraguayan highlands to arid zones, harbour traits for abiotic stress tolerance that could be introgressed into elite lines (Gantait et al., 2018). However, fully harnessing such diversity will require resolving the challenges imposed by the species' reproductive system.

To advance beyond current strategies, we propose an integrative framework (Fig. 2). First, expanding the genetic base through targeted collections in centres of origin, coupled with genomic and metabolomic profiling, enables a comprehensive characterization of available germplasm (Vallejo and Warner, 2021). These data streams (genetic, phenotypic, chemical, phytosanitary) should feed continuously into breeding pipelines to optimize agronomic traits and glycoside profiles. Second, combining *ex situ* and *in situ* conservation ensures that populations can evolve under natural selection and pathogen pressure, yielding more adaptable genetic sources while engaging local communities in areas of original diversity (Benelli *et al.*, 2021).

Third, omic-based approaches, genomics, transcriptomics, metabolomics and phenomics, should be embedded into marker-assisted selection to accelerate the identification of loci controlling key traits (van Der Hooft Jj *et al.*, 2020). These methods can also support the dissection of complex traits related to sexual reproduction and germination performance. Finally, genome-editing tools such as CRISPR/Cas represent a promising frontier not only for enhancing steviol glycoside biosynthesis and stress tolerance but also for resolving reproductive barriers by targeting genes controlling self-incompatibility, embryo development and seed viability (Ghose *et al.*, 2022). Established transformation protocols in stevia already demonstrate that generating transgenic or edited lines is technically feasible (Taak *et al.*, 2020).

While *Stevia rebaudiana* has long been considered a species resistant to full domestication due to its reproductive complexity, emerging molecular technologies open new avenues to overcome these barriers. Future research should explicitly target the genetic and physiological basis of sexual reproduction in stevia, which remains one of the least understood yet most critical aspects limiting its scalable propagation. Addressing this challenge could transform stevia from a semi-domesticated medicinal plant into a fully cultivated, seed-propagated crop.

Conclusions

In this review, we have clarified the breadth of *Stevia*'s genetic diversity, its biotechnological applications, and its commercial relevance. However, realizing these potential hinges on integrating those findings into both breeding programmes and conservation efforts. Beyond its sweetness, *Stevia*'s genetic variability can drive more sustainable cultivation, enhanced disease resistance, improved tolerance to extreme weather stress and novel sensory qualities. Achieving these gains requires integrating in-depth germplasm characterization, dynamic *in situ* and *ex situ* conservation, advanced biotechnological tools and supportive public policies into a cohesive and coherent framework. Only by uniting these elements can *Stevia rebaudiana* evolve from a trending sweetener into a resilient, diverse and long-term sustainable crop.

Crucially, we identified a major biological bottleneck involving photoperiod sensitivity, pollination mechanisms and sexual seed reproduction. Addressing this constraint is fundamental for enabling large-scale stevia cultivation and meeting the growing global demand for steviosides. Future research must prioritize this issue to unlock the species' full reproductive potential and scalability.

Equally important, this integrated framework must operate in parallel with the development of efficient seed-based production systems, including large-scale multiplication of elite, photoperiod-adapted germplasm; optimized agronomic protocols; and high seed quality through standardized postharvest practices. Such progress will require close cooperation between plant breeders and the seed industry to ensure that improved genotypes are multiplied, certified and delivered at scale. Strategic partnerships among public breeding programmes, private seed companies and regulatory institutions will be essential to translate genetic innovation into practical solutions for sustainable stevia cultivation worldwide.

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