

IN VITRO MUTAGENESIS IN CHRYSANTHEMUM: PROTOCOLS, STABILIZATION, AND PROSPECTS FOR CLIMATE RESILIENCE

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ABSTRACT



*In vitro mutagenesis offers an effective approach to improving chrysanthemum (*Chrysanthemum morifolium*), an ornamental crop constrained by polyploidy and complex breeding behaviour. This review examines gamma irradiation and ethyl methanesulfonate (EMS) treatments, mutant stabilization through successive vegetative generations, and molecular validation using ISSR and IRAP markers. Mutagenic responses vary with cultivar, dose, and treatment conditions, influencing survival, regeneration, and desirable phenotypes. Integrating mutagenesis with omics, functional validation, and genome editing could broaden genetic variation and support the development of climate-resilient chrysanthemum cultivars.*

KEYWORDS: Chrysanthemum, Chimaera stabilisation, Climate resilience, Gamma irradiation, In vitro mutagenesis, Molecular markers

INTRODUCTION

Chrysanthemum (*Chrysanthemum morifolium*) is an economically important ornamental crop valued for its diverse flower colours, forms, and commercial applications. Conventional improvement through hybridization and selection is constrained by polyploidy, complex inheritance, and breeding incompatibilities. Induced mutagenesis offers an alternative for generating novel variation, particularly in flower colour, plant architecture, and stress-related traits.

In vitro mutagenesis enables controlled treatment of cultured plant material and facilitates the multiplication and selection of desirable mutants. Gamma irradiation and ethyl methanesulfonate (EMS) are commonly used mutagens. However, successful improvement requires suitable treatment doses, efficient regeneration, stabilization of chimeric shoots, and reliable assessment of genetic variation.

MUTAGENIC TREATMENT PROTOCOLS

1. ***Gamma irradiation:*** Gamma irradiation induces DNA damage and other cellular changes that may generate useful phenotypic variation. The response depends on cultivar, explant type, physiological condition, and radiation dose. The median lethal dose (LD_{50}) is a useful reference for selecting treatment levels, although it must be established experimentally for each material.
The supplied literature reports LD_{50} values around 20–25 Gy for some chrysanthemum cultivars. Lower doses may preserve regeneration capacity, whereas excessive exposure can cause chlorosis, reduced growth, and impaired photosynthesis. Irradiated explants should therefore be evaluated for survival, shoot regeneration, morphological variation, and subsequent flowering performance.
2. ***Ethyl methanesulfonate (EMS):*** EMS is a chemical mutagen that predominantly induces point mutations, including G to A transitions. Concentrations of approximately 0.1–0.5% have been reported for chrysanthemum mutagenesis, but the effective concentration depends on treatment duration, explant characteristics, and cultivar sensitivity. Excessive EMS exposure can reduce survival and regeneration. Preliminary dose-response experiments should determine treatment conditions that balance mutation induction with the recovery of viable plants. Treated material must also be thoroughly rinsed and handled using appropriate chemical-safety procedures.
3. ***Comparative considerations:*** Gamma irradiation can generate conspicuous changes in flower colour and plant architecture, while EMS may produce less visible genetic changes. The effectiveness of either mutagen should be assessed through survival, regeneration, mutation frequency, and stability rather than dose alone. Combined treatments should be considered only after their individual effects and potential interactions have been evaluated.

CHIMERA MANAGEMENT AND MUTANT STABILIZATION

Mutagenesis in chrysanthemum frequently produces chimeras, in which genetically different cell populations coexist within a shoot. Such plants may display altered flower colour or form initially, but these traits can revert or segregate during subsequent growth.

The VM1–VM4 approach involves successive vegetative propagation and selection to identify and stabilize desirable mutants. Repeated nodal or shoot-tip subculturing can help recover genetically uniform shoots, although stabilization is not guaranteed. The process should include careful selection of individual shoots, repeated evaluation of the target phenotype, and confirmation of uniformity across flowering cycles.

Because mutations may affect different meristematic cell layers, visual selection alone may not establish genetic stability. Cytological and molecular assessments can strengthen the evaluation. Stable mutants should ultimately be assessed under production conditions for trait consistency, plant vigour, flowering characteristics, and commercial suitability.

MOLECULAR VALIDATION

Molecular markers can help distinguish putative mutants from untreated plants. Inter-simple sequence repeat (ISSR) and inter-retrotransposon amplified polymorphism (IRAP) markers are useful for detecting DNA polymorphisms and assessing genetic relationships among regenerated plants.

However, marker polymorphism does not necessarily identify the mutation responsible for a particular phenotype or establish that the desired trait will remain stable. Molecular results should therefore be interpreted alongside morphological observations and repeated phenotypic assessments.

Emerging approaches, including single-nucleotide polymorphism (SNP) genotyping, transcriptomics, and metabolomics, can provide additional information. For example, transcriptomic and metabolomic analyses may help investigate changes in pigment biosynthesis associated with flower-colour variation. Combining these methods offers a more comprehensive framework for identifying, validating, and selecting useful mutants.

COMPARATIVE OUTCOMES AND CLIMATE-RESILIENCE APPLICATIONS

Mutagenic outcomes vary considerably with genotype and treatment conditions. The supplied literature reports gamma-irradiation LD₅₀ values of approximately 20–25 Gy and flower-colour mutation frequencies near 2% in some experimental contexts. EMS treatments around 0.2–0.4% have been associated with detectable polymorphism and phenotypic variation. These values are indicative rather than universal recommendations and require confirmation under specific experimental conditions.

Beyond ornamental traits, induced mutagenesis may contribute to climate-resilient breeding by generating variation in heat tolerance, antioxidant responses, and other stress-related characteristics. The supplied manuscript describes heat-tolerant EMS-derived material as a potential avenue for lowland cultivation; however, such claims require validation using accessible primary research evidence.

Future selection programmes should evaluate mutants under relevant heat, drought, and other environmental stresses. Replicated trials across environments are essential to distinguish stable tolerance from temporary responses and to determine whether stress adaptation is maintained without compromising flower quality or yield.

FUTURE PROSPECTS

The integration of in vitro mutagenesis with genomic, transcriptomic, and metabolomic tools can improve the identification and characterization of useful variation. Functional studies can help connect genetic changes with observable traits, while genome editing may provide complementary opportunities for targeted improvement.

Future research should prioritize reproducible treatment protocols, efficient regeneration, reliable chimera stabilization, and rigorous evaluation of stress tolerance. Combining genetic diversity with molecular validation and multi-environment testing could accelerate the development of chrysanthemum cultivars with desirable ornamental characteristics and improved environmental adaptability.

CONCLUSION

In vitro mutagenesis provides a valuable approach to expanding genetic variation in chrysanthemum. Gamma irradiation and EMS can generate useful phenotypic diversity, while successive vegetative propagation and molecular assessment support the identification of stable mutants. Integrating these methods with omics technologies and genome editing may improve selection efficiency and broaden breeding objectives. Nevertheless, cultivar-specific optimization, genetic uniformity, and field validation remain essential for developing commercially viable and climate-resilient chrysanthemum varieties.

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