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GLOBAL RESEARCH LANDSCAPE OF IN VITRO MUTATION BREEDING FOR CROP IMPROVEMENT

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Abstract: *In vitro mutation breeding combines induced mutagenesis with plant tissue-culture systems to expand genetic variation while enabling selection and regeneration under controlled conditions. This study mapped the global research landscape of in vitro mutation breeding for crop improvement using Scopus-indexed literature. The initial search retrieved 635 records; after restriction to Agricultural and Biological Sciences, journal articles, exclusion of the incomplete 2026 publication year, topical screening, and duplicate removal, 182 articles published between 1966 and 2025 were retained. Performance analysis assessed publication growth, leading sources, authors, countries, and citation impact, while science mapping was performed with VOSviewer. The keyword co-occurrence network comprised 48 terms organized into four clusters, with 516 links and a total link strength of 882. The conceptual structure was centered on mutation, mutagenesis, mutation breeding, tissue culture, and in vitro approaches, with application-oriented subthemes involving stress tolerance, disease resistance, banana, potato, and chrysanthemum. Overlay visualization suggested a progression from classical irradiation- and regeneration-centered research toward greater emphasis on genetic variation, DNA-level characterization, polymorphism, and chemical mutagenesis. These findings show that in vitro mutation breeding has evolved from a specialized experimental approach into a connected research field integrating mutation induction, regeneration, trait selection, and molecular characterization.*

Keyword: bibliometric analysis; co-occurrence mapping; in vitro mutagenesis; research trends; tissue culture

INTRODUCTION

Crop improvement depends on the availability of heritable genetic variation from which useful phenotypes can be selected. Induced mutation breeding expands this variation by exposing plant material to physical or chemical mutagens and subsequently identifying desirable mutants. Mutation-derived varieties have made a documented contribution to global crop improvement, particularly where breeders seek to modify one or a few traits while retaining an otherwise adapted genetic background (Ahloowalia et al., 2004). International programmes have also established induced mutation as a practical complement to conventional breeding, with applications spanning yield, quality, disease resistance, abiotic-stress tolerance, and ornamental traits (Jain, 2005; Maluszynski et al., 1995; Shu, 2009; Shu et al., 2012).

The combination of mutagenesis with in vitro culture offers additional advantages. Tissue-culture systems provide controllable explants, facilitate treatment of large cell or tissue populations, allow selection under defined conditions, and can accelerate regeneration of putative mutants. These features are especially relevant for vegetatively propagated crops, ornamentals, and genotypes in which sexual crossing is difficult or slow. Early and subsequent studies demonstrated the practical integration of adventitious-bud culture, somatic embryogenesis, callus systems, and other in vitro platforms with mutation induction in potato (van Harten et al., 1981), chrysanthemum (Broertjes et al., 1976), grapevine (Kuksova et al., 1997), maize (Novak et al., 1988),

cassava (Joseph et al., 2004), banana (Bhagwat & Duncan, 1998), sugarcane (Patade et al., 2008), and rice (Chen et al., 2001). Physical mutagens such as gamma rays, X-rays, heavy-ion beams, and other ionizing radiation, as well as chemical mutagens such as ethyl methanesulfonate (EMS), have been used across these systems, although biological response varies with genotype, explant type, dose, exposure duration, and regeneration pathway (Oladosu et al., 2016; Shu et al., 2012; Yamaguchi et al., 2008).

Recent crop-focused protocols make this in vitro component explicit: induced mutation is coupled to cultured explants, somatic embryogenesis, micropropagation, or recovery of regenerants in raspberry, sweet potato, potato, rice, banana, and citrus (Dai & Zhang, 2025; Dang et al., 2025; Kaur et al., 2024; Murugan et al., 2024; Pérez et al., 2024; Rajan et al., 2023). Comparable evidence from ornamental breeding links mutation treatment to cultured buds, protocorm-like bodies, or callus followed by plantlet regeneration and phenotypic or molecular assessment (Haspolat, 2022, 2024; Martha et al., 2023; Maulida et al., 2025). Together, these studies distinguish tissue-culture-based breeding from experiments that merely use in vitro laboratory assays to measure a plant trait, and show that the experimental role of tissue culture, rather than the appearance of the phrase in vitro in an abstract, defines the field.

In vitro systems can serve as both the biological material exposed to mutagens and the platform through which mutants are recovered and selected. Callus, adventitious shoots, somatic embryos, meristems, anthers, floral tissues, and other explants permit controlled exposure and large-scale handling, while culture media can be modified to impose salinity, disease-related, or other selection pressures (Chen et al., 2001; Joseph et al., 2004; Latado et al., 2004; Patade & Suprasanna, 2009). However, the same systems can generate somaclonal variation and chimerism, so treatment-induced variants must be distinguished from culture-derived changes through careful regeneration, stabilization, and phenotypic or molecular validation (Cassells et al., 1997; Chakravarty & Sen, 2001; Gavazzi et al., 1987; Kuksova et al., 1997; Mandal et al., 2000; Novak et al., 1988; Oladosu et al., 2016).

The value of the approach therefore depends not only on mutation induction but also on the ability to recover, screen, stabilize, and validate variants. Marker-assisted assessment has been used to distinguish induced variation and characterize regenerated lines in potato (Yaycili & Alikamanoğlu, 2012), sugarcane (Khan et al., 2009), Etlingera (Yunus et al., 2013), Miscanthus (Perera et al., 2015), chrysanthemum (Nasri et al., 2022), and wheat (Türkoğlu et al., 2023). Recent reviews continue to identify integration with molecular and genomic tools, improved dosimetry, and robust phenotypic validation as priorities for effective crop improvement (Bado et al., 2017; Sharma et al., 2025). These syntheses also separate mutation-induction technologies, regeneration systems, and subsequent trait or molecular validation, and they situate induced mutagenesis alongside, but conceptually apart from, targeted genome engineering (Ali & Talekar, 2024; Animasaun & Oguntoye, 2024; Bharath et al., 2024; Guo et al., 2024; Shahwar et al., 2023).

Despite decades of experimentation, the literature is fragmented across crop species, mutagens, tissue-culture systems, and target traits. Conventional narrative reviews are useful for summarizing mechanisms and applications but are less suited to quantifying how a research field has evolved, which sources and countries have shaped it, and how research topics cluster and change over time. Bibliometric analysis provides a reproducible framework for answering these questions through performance analysis and science mapping (Donthu et

al., 2021). The approach has already been applied to related questions; for example, a Scopus-based analysis of 190 articles on gamma-ray mutagenesis in forage crops published between 2010 and 2023 identified mutation induction, DNA repair, and radiation response as dominant themes (Putra et al., 2025). Such studies, however, focus on a single mutagen or crop group and do not isolate the in vitro component of mutation breeding. To the author's knowledge, no bibliometric study has yet mapped tissue-culture-based mutation breeding across crops, mutagens, and target traits.

Accordingly, this study provides a Scopus-based bibliometric assessment of in vitro mutation breeding for crop improvement. It aims to (i) characterize the temporal growth and citation profile of the literature; (ii) identify the most productive sources, authors, and countries; (iii) determine influential documents within the corpus; and (iv) map the principal conceptual themes using author-keyword co-occurrence. By using a manually screened corpus, the analysis also addresses a recurring retrieval problem in this topic: generic terms such as mutation, plant, and in vitro can capture genome-editing, microbial, human, or regeneration-only studies that are not substantively part of mutation breeding.

METHODS

Study design and data source. A bibliometric design was used to characterize the development and conceptual structure of research on in vitro mutation breeding for crop improvement. Scopus was selected as the bibliographic source because it provides structured records for titles, abstracts, author and indexed keywords, affiliations, cited-by counts, and cited references suitable for bibliometric analysis. The choice of a single database also improves metadata consistency, although it limits coverage to the journals and document types indexed by that database. Database coverage can differ across bibliographic platforms, and this limitation is considered in the interpretation of results (Falagas et al., 2008).

Search strategy. The search was conducted on 14 October 2026 in the Scopus title, abstract, and keyword fields. The reported Boolean concept structure was: ("induced mutation" OR "induced mutagenesis" OR "mutation breeding") AND ("in vitro" OR "tissue culture" OR callus OR explant) AND (plant OR plants OR crop OR crops OR cultivar OR breeding). Subject-area and document-type restrictions were applied after initial retrieval, as described below.

The initial query returned 635 records. Restriction to the Scopus subject area Agricultural and Biological Sciences reduced the set to 442 records. Limiting the document type to Article produced 285 records. No language restriction and no open-access restriction were applied.

Eligibility criteria, screening, and data cleaning. Publications from 2026 (n = 18) were removed because the retrieval date preceded completion of the calendar year. Titles and abstracts of the remaining records were screened for topical relevance. Eligible publications addressed induced mutation or mutation breeding in plants and incorporated an in vitro-related component, including tissue culture, callus culture, explants, somatic tissues, in vitro regeneration, or in vitro selection in the context of crop or plant improvement. Exclusions comprised targeted genome-editing studies (e.g., CRISPR/Cas, TALEN, or zinc-finger nuclease studies), microbial/animal/human mutagenesis, genotoxicity or antimutagenicity assays unrelated to plant breeding, regeneration-only studies without induced mutagenesis, and general biotechnology articles in which mutation breeding was peripheral. Eighty-four topically irrelevant records and one duplicate were removed, leaving a final analytical corpus of 182 publications from 1966–2025. The bibliometric record-selection process is

summarized in Figure 1. The record-selection process was documented using an adapted PRISMA 2020 flow-diagram framework to improve transparency and reproducibility of bibliometric screening (Page et al., 2021). Bibliographic metadata were checked for duplicate records and obvious inconsistencies before analysis. One publication-year discrepancy was corrected using publisher metadata to ensure accurate temporal analysis.

For interpretation of the eligibility boundary, an in vitro component refers to the culture, treatment, selection, or regeneration of plant cells, tissues, or explants as part of mutation breeding. An assay performed in vitro solely to quantify a trait does not, by itself, establish an in vitro mutation-breeding experiment. Reproducible bibliometric screening requires clearly documented inclusion decisions, especially for boundary cases and broadly worded keyword matches (Du et al., 2024; Lim et al.,2024).

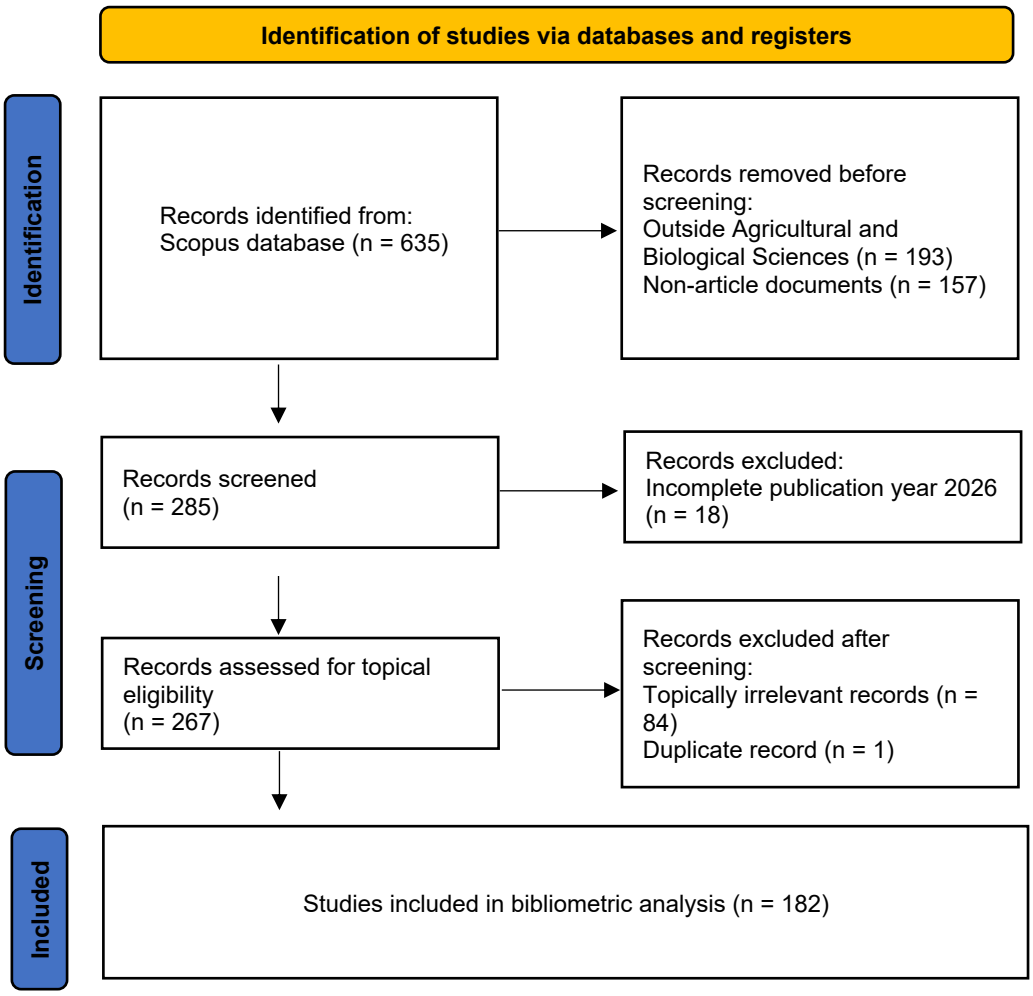


Figure 1. Record identification, screening, and inclusion process for the bibliometric analysis.
Adapted from the PRISMA 2020 flow-diagram framework (Page et al., 2021); used here to transparently report bibliometric record selection.

Bibliometric indicators and network analysis. Performance analysis covered annual scientific production, source productivity, author productivity, affiliation-based country contribution, citation counts, and the most-cited

documents. Science mapping focused on keyword co-occurrence to identify the conceptual structure and thematic relationships within the corpus. VOSviewer version 1.6.21 was used to construct network, overlay, and density visualizations. In the resulting co-occurrence map, node size represents term occurrence, link lines represent co-occurrence relationships, and spatial proximity reflects relatedness. VOSviewer clustering was used to identify groups of strongly connected terms. The final map contained 48 terms grouped into four clusters, with 516 links and a total link strength of 882.

Before interpretation, obvious lexical and semantic variants were considered during data cleaning and map review (for example, EMS versus ethyl methanesulfonate, gamma rays versus gamma radiation/gamma irradiation, banana versus Musa, and potato versus Solanum tuberosum). Because VOSviewer can retain related variants as separate terms when they occur independently in the source metadata, the submitted maps were interpreted at the thematic level rather than treating every lexical variant as a distinct scientific concept. Cluster labels were assigned inductively from the most visible and conceptually coherent terms in each group.

Reproducibility and software. Data cleaning and descriptive analysis were conducted programmatically from the Scopus CSV export. Network visualization and science mapping were conducted in VOSviewer 1.6.21, following established principles for bibliometric mapping (van Eck & Waltman, 2010). The analytical workflow follows the distinction between performance analysis and science mapping described by Donthu et al. (2021), while Bibliometrix/Biblioshiny provides a complementary reproducibility framework for bibliometric data handling and descriptive checks (Aria & Cuccurullo, 2017). All numerical results reported in the manuscript were derived from the cleaned corpus, and the VOSviewer maps supplied for final analysis were used as the authoritative source for network statistics and cluster structure.

RESULT AND DISCUSSION

Corpus characteristics and temporal development.

The final corpus contained 182 articles published from 1966 through 2025 in 101 source titles. The articles accumulated 3,348 Scopus citations, with a mean of 18.4 and median of 8 citations per article. Fifteen articles had not yet been cited at the retrieval date, whereas 85 had received at least 10 citations and 17 had received at least 50 citations. The corpus involved 763 distinct exported author-name strings, with a mean of 4.69 authors per paper; 176 papers (96.7%) were multi-authored.

Scientific output was limited during the early decades but expanded markedly after 2010. Only 23 articles were published through 1999 and 27 during 2000–2009, compared with 74 during 2010–2019 and 58 during the six-year period 2020–2025. The annual maximum occurred in 2025 with 14 publications, indicating continued activity at the end of the observation period.

The temporal profile indicates that in vitro mutation breeding has moved from a relatively specialized technique to a sustained research stream. The expansion after 2010 likely reflects several converging factors: continued use of mutation breeding in crop diversification, improved tissue-culture and regeneration protocols, greater access to molecular characterization, and renewed demand for stress-tolerant and quality-improved crops. This trajectory is consistent with the broader evolution of mutation breeding from phenotype-driven selection toward integration with molecular and genomic tools described in international reference works (Shu, 2009; Shu et al., 2012). This continuity is also visible in

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the corpus itself, which ranges from early adventitious-bud mutation systems in ornamentals and potato to recent molecularly characterized EMS and irradiation studies (Broertjes et al., 1976; Nasri et al., 2022; Sharma et al., 2025; Türkoğlu et al., 2023; van Harten et al., 1981). The annual publication pattern and the distribution of articles across broad publication periods are shown in Figure 2 and Table 1, respectively.

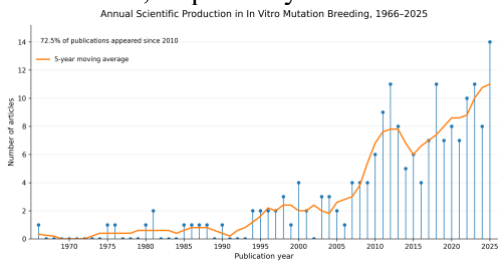


Figure 2. Annual scientific production in the final corpus, 1966–2025. Source: Author's analysis of the cleaned Scopus dataset.

Table 1. Publication output by broad period

Period	Articles	Share of corpus
1966–1999	23	12.6%
2000–2009	27	14.8%
2010–2019	74	40.7%
2020–2025	58	31.9%

Source: Author's analysis of the cleaned Scopus dataset.

Leading publication sources.

The literature was distributed across 101 source titles, indicating substantial disciplinary dispersion. Nevertheless, a small group of plant-breeding and tissue-culture journals accounted for a notable share of output. Euphytica ranked first with 14 articles, followed by Plant Cell, Tissue and Organ Culture with 13. The remaining leading sources each contributed fewer than six articles, underscoring the long-tail distribution of the literature. The ten most productive publication sources are presented in Figure 3 and summarized in Table 2.

The leading journals demonstrate the dual disciplinary identity of the field. Euphytica represents the plant-breeding side, whereas Plant Cell, Tissue and Organ Culture represents the regeneration and in vitro biotechnology side. Their prominence supports the conceptual premise that successful in vitro mutagenesis is not only a mutagen-treatment problem: it also depends on reliable regeneration, selection, and stabilization of variants. This is particularly important where chimerism, somaclonal variation, or genotype-dependent regeneration can complicate attribution of the final phenotype to the intended mutagenic treatment.

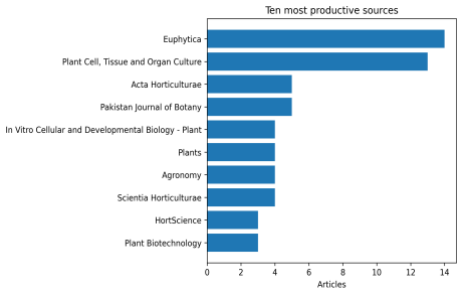


Figure 3. Ten most productive publication sources. Source: Author's analysis of the cleaned Scopus dataset.

Table 2. Ten most productive sources

Rank	Source	Articles	Share
1	Euphytica	14	7.7%
2	Plant Cell, Tissue and Organ Culture	13	7.1%
3	Pakistan Journal of Botany	5	2.7%
4	Acta Horticulturae	5	2.7%
5	In Vitro Cellular and Developmental Biology - Plant	4	2.2%
6	Plants	4	2.2%
7	Agronomy	4	2.2%
8	Scientia	4	2.2%

	Horticulturae		
9	HortScience	3	1.6%
10	Plant Biotechnology	3	1.6%

Productive authors, geographic distribution, and collaboration.

Author productivity was highly dispersed. The most frequent author name strings were Gatica-Arias A. and Suprasanna P., with five articles each, followed by Miler N., Broertjes C., and Abe T. with four each. Because Scopus author strings may contain variants and because this analysis was based on exported names rather than ORCID-level disambiguation, these values should be interpreted as publication counts attached to the exported author identities. The most productive author name strings are summarized in Table 3.

Affiliation-based counting showed a broad international distribution. India contributed to 42 articles, followed by China (17) and Turkey (11). Japan contributed to nine publications, and Poland to eight. Twenty-three articles (12.6%) contained affiliations from at least two countries, suggesting that most publications were produced within single-country teams, despite the global spread of the topic. Because country counts are non-exclusive, totals across countries exceed the number of papers. The geographic distribution of the leading contributing countries is illustrated in Figure 4 and detailed in Table 4.

India's leading publication count, followed by China and Turkey, suggests strong activity in countries with substantial plant-breeding and tissue-culture communities. However, only 12.6% of papers contained affiliations from more than one country. Greater international collaboration could be particularly valuable for mutation breeding because reproducibility depends on access to standardized mutagen facilities, dosimetry, tissue-culture expertise, molecular validation, and multi-environment testing. Cross-country networks could also help move promising in vitro mutants beyond proof-of-concept studies toward stable, agronomically validated breeding material.

Table 3. Most productive author name strings

Rank	Author	Articles
1	Gatica-Arias A.	5
2	Suprasanna P.	5
3	Miler N.	4
4	Broertjes C.	4
5	Abe T.	4
6	Sharma A.	3
7	Kumar P.	3
8	Gosal S.S.	3
9	Encheva J.	3
10	Haspolat G.	3

Source: Author's analysis of the cleaned Scopus dataset.

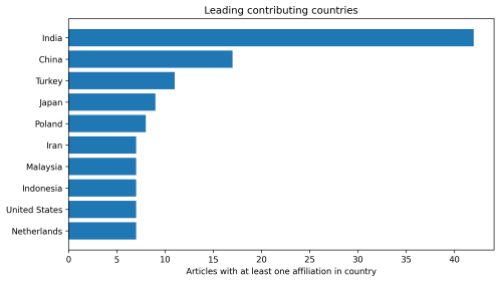


Figure 4. Ten leading countries by affiliation-based publication count. Source: Author's analysis of the cleaned Scopus dataset.

Table 4. leading countries by affiliation count

Rank	Country	Articles
1	India	42
2	China	17
3	Turkey	11
4	Japan	9
5	Poland	8
6	Iran	7
7	Malaysia	7
8	Indonesia	7
9	United States	7
10	Netherlands	7
11	Thailand	7
12	Austria	6
13	Italy	6
14	Pakistan	6
15	Costa Rica	5

Source: Author's affiliation parsing of the cleaned Scopus dataset; counts are non-exclusive.

Citation profile and influential documents.

Citation impact was unevenly distributed. The most-cited document in the corpus was the 1995 Euphytica article "Application of in vivo and in vitro mutation techniques for crop improvement" (205 citations), followed by "Chrysanthemum Biotechnology: Quo vadis?" (122 citations) and "Major mutation-assisted plant breeding programs supported by FAO/IAEA" (119 citations). Older documents naturally had longer citation windows; therefore, the ranking should not be interpreted as a direct measure of current methodological relevance. Table 5 summarizes the ten most-cited documents in the analytical corpus, together with their publication year, source title, and Scopus citation count.

Table 5. Ten most-cited documents within the analytical corpus

Rank	Document title	Year	Source	Scopus citations
1	Application of in vivo and in vitro mutation techniques for crop improvement (Maluszynski et al., 1995)	1995	Euphytica	205
2	Chrysanthemum Biotechnology: Quo vadis? (Teixeira da Silva et al., 2013)	2013	Critical Reviews in Plant Sciences	122
3	Major mutation-assisted plant breeding programs supported by FAO/IAEA (Jain, 2005)	2005	Plant Cell, Tissue and Organ Culture	119
4	Effects of dose and dose rate of gamma ray irradiation on mutation induction and nuclear DNA content in chrysanthemum (Yamaguchi et al., 2008)	2008	Breeding Science	76
5	In vitro selection and identification of sweetpotato (*Ipomoea batatas* (L.) Lam.) plants tolerant to NaCl (He et al., 2009)	2009	Plant Cell, Tissue and Organ Culture	75
6	Somaclonal variation versus chemically induced mutagenesis in tomato (*Lycopersicon esculentum* L.) (Gavazzi et al., 1987)	1987	Theoretical and Applied Genetics	74
7	Mutation breeding of *Chrysanthemum morifolium* Ram. using in vivo and in vitro adventitious bud techniques (Broertjes et al., 1976)	1976	Euphytica	74
8	Mutation breeding of banana cv. Highgate (*Musa* spp., AAA Group) for	1998	Scientia Horticultu	71

	tolerance to *Fusarium oxysporum* f. sp. cubense using chemical mutagens (Bhagwat & Duncan, 1998)		rae	
9	Development of EMS-induced mutation population for amylose and resistant starch variation in bread wheat (*Triticum aestivum*) and identification of candidate genes responsible for amylose variation (Mishra et al., 2016)	2016	BMC Plant Biology	70
10	Mutation induced by ethylmethanesulphonate (EMS), in vitro screening for salt tolerance and plant regeneration of sweet potato (*Ipomoea batatas* L.) (Luan et al., 2007)	2007	Plant Cell, Tissue and Organ Culture	

Source: Scopus cited-by counts in the export retrieved 14 October 2026; citation windows differ by publication year.

Keyword structure, research themes, and conceptual evolution.

The VOSviewer keyword co-occurrence analysis identified a densely interconnected conceptual structure. The final network contained 48 terms distributed across four clusters, connected by 516 links with a total link strength of 882. The largest and most visually prominent nodes included mutation, mutagenesis, mutation breeding, tissue culture, in vitro, gamma irradiation, and chrysanthemum. Their central positions indicate that the literature is organized around a common core linking induced genetic variation with tissue-culture-mediated regeneration and plant-breeding applications. The overall keyword co-occurrence structure is visualized in Figure 5, while the four thematic clusters and their representative terms are summarized in Table 6.

The VOSviewer network confirms the persistent importance of both physical and chemical mutagens. Gamma irradiation, gamma rays, and gamma radiation occupy prominent positions around the in vitro and tissue-culture core, whereas EMS/ethyl methanesulfonate and sodium azide appear within chemical-mutagen themes. Their placement across interconnected clusters suggests that mutagen choice is closely tied to explant type, regeneration capacity, crop species, and target trait rather than representing separate methodological traditions. This pattern is consistent with the long-standing complementary use of physical and chemical mutagens in crop improvement (Oladosu et al., 2016; Shu et al., 2012).

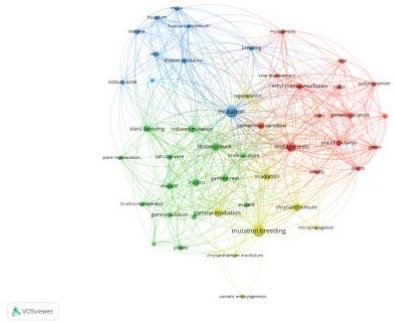


Figure 5. VOSviewer network visualization of keyword co-occurrence. Source: Author's analysis using VOSviewer 1.6.21. Network statistics: 48 items, 4 clusters, 516 links, total link strength = 882.

Table 6. Interpreted keyword communities

Cluster	Interpretive theme	Representative keywords
Cluster 1	Genetic variation, mutagenesis, and crop	mutagenesis; genetic variation;

	improvement	DNA; genes; polymorphism; callus; ethyl methanesulfonate; salinity; crop improvement
Cluster 2	In vitro culture, induced mutation, and stress-oriented breeding	tissue culture; in vitro; in vitro culture; in vitro mutagenesis; induced mutation; mutant; gamma irradiation; plant breeding; plant regeneration; salt tolerance; potato
Cluster 3	Banana breeding, disease resistance, and chemical mutagens	banana; *Musa*; *Fusarium*; *Fusarium oxysporum*; disease resistance; breeding; EMS; sodium azide
Cluster 4	Mutation breeding and ornamental/horticultural regeneration	mutation breeding; chrysanthemum; *Chrysanthemum morifolium*; irradiation; regeneration; micropropagation; somatic embryogenesis

The four clusters indicate distinct but strongly connected research domains. Cluster 1 represents genetic variation, mutagenesis, and crop improvement, linking terms such as mutagenesis, genetic variation, DNA, genes, polymorphism, callus, ethyl methanesulfonate, and salinity. Cluster 2 captures the methodological core of in vitro mutation breeding, including tissue culture, in vitro culture, in vitro mutagenesis, induced mutation, gamma irradiation, plant regeneration, plant breeding, potato, and salt tolerance. Cluster 3 is application-specific, centering on banana/Musa, Fusarium and Fusarium oxysporum, disease resistance, EMS, and sodium azide. Cluster 4 emphasizes mutation breeding in ornamental and horticultural systems, particularly chrysanthemum, irradiation, regeneration, micropropagation, and somatic embryogenesis. The extensive cross-cluster linkages show that mutagen treatment, regeneration, molecular characterization, and trait selection are not isolated themes but components of an integrated research system.

The co-occurrence clusters and overlay map indicate a progression from mutation induction as an experimental endpoint toward mutation breeding embedded within trait-selection and molecular-characterization pipelines. Salt tolerance and salinity connect strongly with in vitro culture and induced mutation, while disease resistance links a distinct banana-Musa-Fusarium theme with EMS and sodium azide. At the same time, relatively later terms such as DNA, genes, polymorphism, and genetic variation signal increasing use of molecular evidence to validate and characterize induced variability. Thus, the field appears to be evolving from predominantly phenotype-based mutant recovery toward integrated workflows combining mutation induction, regeneration, trait screening, and genetic confirmation.

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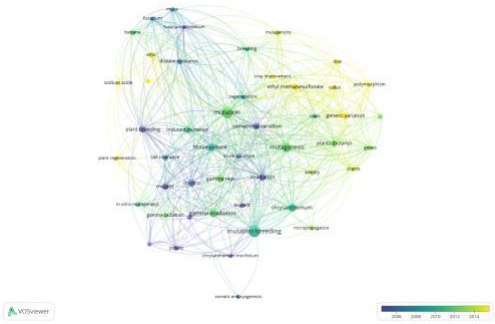


Figure 6. VOSviewer overlay visualization of keyword co-occurrence by average publication year. Source: Author's analysis of the cleaned Scopus dataset using VOSviewer 1.6.21.

The overlay visualization suggests temporal differences in keyword emphasis within the retrieved corpus. As shown in Figure 6, earlier research is more strongly associated with plant breeding, mutant, in vitro, gamma radiation, potato, and related regeneration-oriented terms. Relatively later-emerging terms include DNA, genes, polymorphism, genetic variation, callus, mutagenicity, and ethyl methanesulfonate. This pattern suggests a transition from classical irradiation and phenotypic selection toward greater integration of molecular characterization and chemically induced variation.

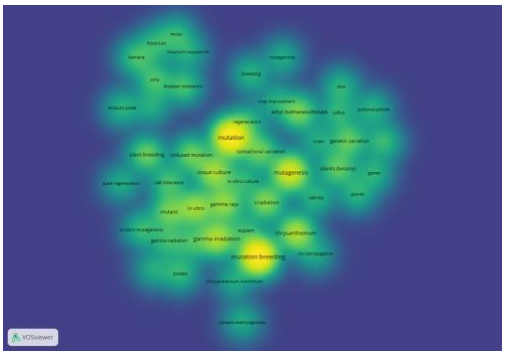


Figure 7. VOSviewer density visualization of keyword co-occurrence. Source: Author's analysis of the cleaned Scopus dataset using VOSviewer 1.6.21.

The density visualization identifies mutation, mutagenesis, mutation breeding, and tissue culture as the principal conceptual hotspots. Around this core, application-oriented terms such as banana, Fusarium, chrysanthemum, potato, salinity, and disease resistance form secondary concentrations, demonstrating that the field combines a stable methodological foundation with crop- and trait-specific applications.

Taken together, Figures 5–7 show a field with a stable methodological core and a gradually broadening analytical frontier. The central hotspot is formed by mutation, mutagenesis, mutation breeding, tissue culture, and in vitro approaches. Crop-specific themes, particularly banana-Fusarium disease resistance, potato-salinity tolerance, and chrysanthemum regeneration, radiate from this core. The overlay visualization further suggests that molecular terms became relatively more prominent later than classical plant-breeding and irradiation terminology. This evolution supports a future research agenda in which induced variation is combined with standardized regeneration protocols, high-throughput molecular characterization, robust phenotyping, and multi-environment validation of mutant lines.

Research gaps, future directions, and limitations.

Several research gaps remain in the field of in vitro mutation breeding. Future studies should adopt more standardized reporting of mutagen exposure, including dose or concentration, exposure duration, explant type and physiological state, survival, regeneration response, and the rationale for selecting treatment levels, as this would improve comparability across studies. Greater attention is also needed to distinguish mutagen-induced variation from somaclonal variation. Appropriate regeneration controls and, where feasible, molecular validation should therefore be incorporated to clarify whether observed phenotypic changes are associated with the mutagenic treatment or arise from the tissue-culture process itself. In addition, mutation stability and field performance require more systematic evaluation,

because many in vitro studies terminate at the regeneration or early phenotyping stage. Multi-generation or clonal stability assessments, together with field evaluation, are necessary before mutant lines can be considered relevant for breeding programs. The integration of molecular and genomic validation through marker systems, sequencing, and functional assays may further strengthen causal interpretation and improve the efficiency of mutant selection without shifting the approach toward targeted genome editing. Greater international and multi-environment collaboration is also needed, particularly because the relatively low proportion of internationally co-authored publications suggests opportunities for more extensive sharing of protocols, facilities, and validation across environments. Future bibliometric studies should likewise strengthen reproducibility by transparently reporting search strategies, screening criteria, author and keyword disambiguation procedures, and VOSviewer or Bibliometrix parameters.

This study also has several limitations. First, the analysis relied exclusively on Scopus; therefore, publications indexed only in other bibliographic databases were not captured. Second, restricting the search to the Agricultural and Biological Sciences subject area improved topical precision but may have excluded relevant cross-disciplinary studies assigned solely to other subject categories. Third, bibliometric retrieval is sensitive to terminology, and older or crop-specific studies may not use the exact terms included in the search query. Fourth, title and abstract screening necessarily involved a degree of judgment despite the use of explicit eligibility criteria. Fifth, citation-based indicators are influenced by publication age, meaning that older articles generally have had more time to accumulate citations. Sixth, author and institutional disambiguation based on exported Scopus metadata cannot fully resolve name variants or organizational changes. Finally, the VOSviewer keyword map retained several semantically overlapping terms, such as gamma rays, gamma radiation, and gamma irradiation, as well as EMS and ethyl methanesulfonate. Consequently, the interpretation of thematic clusters emphasized broader conceptual patterns rather than treating every lexical variant as a distinct scientific concept.

CONCLUSION

This bibliometric analysis identified 182 Scopus-indexed articles on in vitro mutation breeding for crop improvement published between 1966 and 2025. Research output increased substantially after 2010, with the highest annual publication count observed in 2025. The field combines induced mutagenesis, tissue-culture regeneration, trait-based selection, and increasingly prominent molecular characterization. Gamma irradiation and EMS remain important mutagenic approaches, while salt tolerance, disease resistance, banana, potato, and chrysanthemum constitute recurrent applied themes. Euphytica and Plant Cell, Tissue and Organ Culture were the most productive sources; India, China, and Turkey were the leading affiliation-based contributors. The limited proportion of internationally co-authored articles indicates scope for wider collaboration. Overall, the maps highlight a connected research landscape in which mutation induction alone is insufficient: reliable regeneration, mutation-stability assessment, molecular validation, and multi-environment phenotyping are essential for translating in vitro variants into useful breeding material. The findings offer a baseline for subsequent bibliometric updates, provided that the cleaned records and full mapping parameters are made available for independent verification.

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