Biotechnological Advances in Brassicaceae Crops

Ruchira Gurav*, Himanshu Dawda, Usha Mukundan

Plant Biotechnology Research Lab, Ramniranjan Jhunjhunwala College (Empowered Autonomous), Ghatkopar (West), Mumbai – 400086, Maharashtra, India.

*Corresponding Author Email – ruchira95gurav@gmail.com

Abstract

Brassicaceae crops are well known for their nutritional value, phytochemical content, and health benefits. Traditional growing practices for these crops are often affected by long generation times, environmental effects, and complex genome structures. Advancements in *in vitro* regeneration systems, from classical tissue culture techniques to modern molecular approaches are summarised in this review. Major regeneration pathways, such as organogenesis, somatic embryogenesis, callus culture, microspore culture, and doubled haploid (DH) production, are discussed in the context to mass propagation, genetic improvement, and secondary metabolite production. The review summarises biotechnological milestones, recent developments, current challenges and prospects, emphasising the integration of tissue culture and advanced biotechnology for sustainable brassicaceae crop improvement.

Keywords: Brassicaceae, plant tissue culture, *in vitro* regeneration, secondary metabolites, organogenesis, somatic embryogenesis

Abbreviations

$AgNO_3$ – Silver nitrate

BA – 6-Benzyladenine

BAP – 6-Benzylaminopurine

BolMYB28 – *Brassica oleracea* MYB Domain Protein 28 (R2R3-MYB transcription factor)

cDNA – Complementary DNA

Cas – CRISPR-associated protein

CRISPR – Clustered Regularly Interspaced Short Palindromic Repeats

DH – Doubled haploid

DNA – Deoxyribonucleic acid

FAD2 – Fatty Acid Desaturase 2

FAE1 – Fatty Acid Elongation 1

GSL – Glucosinolate(s)

IBA – Indole-3-butyric acid

MeJA – Methyl jasmonate

NAA – α-Naphthaleneacetic acid

PGR – Plant Growth Regulator(s)

RNA – Ribonucleic acid

RNP – Ribonucleoprotein

TDZ – Thidiazuron

1. Introduction

Brassicaceae is one of the largest angiosperm families within the order Brassicales. It consists of nearly 338 genera and 3709 species. It has diverse group of plants, from food crops to ornamental species found all around the world, except in Antarctica. They are more prevalent in regions such as Asia, North America, and Europe. These plants have adapted to various climates and ecosystems, making them a widely distributed family (Al-Shehbaz et al., 2006). *Brassica* crops show great versatility and are mainly cultivated in temperate regions across the globe (Sjödin, C; 1992). Within the family, we can find a variety of plants, including annuals, biennials, and herbaceous perennials. Most of them are characterised as herbs, constituting the predominant portion, with only a small fraction (approximately 5%) exhibiting traits of woody growth (Raza et al., 2020).

The pungent smell from the crushed leaves of Brassicaceae plants is among the distinctive characteristic linked to the family. They have a watery sap that contains the non-poisonous glucosinolates. Taproot is the main root system of these plants. Leaves are often in a basal rosette while stem leaves are alternate or rarely opposite when present. The flowers are mostly bisexual; radially symmetrical, grouped in racemes or in solitary on pedicels that emerge from basal rosettes. The four sepals are rarely united and alternate with four petals forming a cross known a cruciform corolla giving the family name '*Cruciferae*'. (Raza A. et al., 2020) (Al-Shehbaz 2011).

2. Economically Important Brassicaceae Vegetables

Brassica vegetables are an important and highly diversified group of crops grown worldwide and belong mainly to the species *B. oleracea*, as well as *B. rapa* and *B. napus*. The *Brassica* genus encompasses, but is not confined to, a variety of vegetables, such as Bok choy, Broccoli, Brussels sprouts, Cabbage, Cauliflower, Chinese cabbage, Kale, Kohlrabi, Mizuna, and Turnips. Furthermore, there are other closely related vegetables within the Brassicaceae family also recognised as *brassica* or cruciferous vegetables; which mainly includes Radish (*Raphanus sativus*), Watercress (*Nasturtium officinale*), Arugula (*Eruca sativa*), Horseradish (*Armoracia rusticana*), Maca (*Lepidium meyenii*), Mashua (*Tropaeolum tuberosum*), Wasabi (*Wasabia japonica*), and Cress (*Lepidium sativum*) (Fahey, J. W. 2015) (Jong-In Park et al., 2012).

3. Nutritional and Phytochemical Significance of Brassicaceae

Brassica vegetables contain little fat, and are sources of vitamins, minerals, and fibre. They also contain many novel phytochemicals, some of which offer protection against carcinogenesis (Jong-In Park et al., 2012). Members of the family serve as significant suppliers of bioactive substances and essential nutrients such as Vitamin E and C, soluble fibre, and enzymes that contribute to their antioxidant properties. Enzymes include peroxidase, superoxide dismutase, and catalase. Additionally, these plants contain carotenoids that display compelling antiviral, antibacterial, and anticancer effects (Sharma, P. et al., 2015).

4. Secondary Metabolites and Their Biological Role

Many phytochemicals have antioxidant activity, due to their hydrogen-donating and reducing properties. Antioxidants are oxidised themselves while neutralising reactive oxidising agents and thus protect biomolecules from oxidative damage. Secondary metabolites are also important in plant defence against pathogens and weeds. These metabolites are often species-, genus- and family-specific in Brassicaceae plants and include glucosinolates, oils and seed fatty acids (Fahey et al., 2001; Raza et al., 2020).

Brassicaceae vegetables are also sources of phenolic acids and flavonoids (quercetin, kaempferol, sinapic acid and ferulic acid) in addition to glucosinolates. These compounds possess antioxidant activity and can scavenge free radicals, chelate metal ions and inhibit lipid peroxidation (Cartea et al., 2010). A positive correlation has been reported between phenolic content and antioxidant capacity for several Brassica vegetables such as broccoli, cabbage, kale, mustard greens and cauliflower.

Nutritionally, Brassicaceae are valued for their high levels of vitamins and minerals, such as vitamins C, E and K, folates, calcium, potassium, magnesium and dietary fibre. Carotenoids (e.g. β-carotene, lutein and zeaxanthin) are also involved in eye health and antioxidant defence mechanisms (Podsędek, 2007). Brassica vegetables are considered functional foods due to their health benefits; regular consumption reduces the risks of obesity, cardiovascular diseases, diabetes and some cancers.

Recent breeding and biotechnological advances have been aimed at improving the nutritional quality and phytochemical composition of Brassicaceae crops. There are considerable genetic differences in glucosinolate profile, antioxidant capacity and accumulation of bioactive compounds among different Brassica species and cultivars (Raza et al., 2020).

5. Need for Biotechnological Intervention in Brassicaceae

An increase in consumer awareness about healthy dietary habits and prevention of lifestyle-associated diseases (Avato & Argentieri, 2015) has led to a significant increase in the market demand for Brassicaceae vegetables all over the world. Thus, the rising consumer demand calls for the increased necessity of improving crop productivity and nutritional quality.

Although Brassicaceae crops are of nutritional importance, their production and supply are challenged by several factors. The conventional cultivation practices are largely linked with reduced productivity due to susceptibility to biotic stresses such as fungal, bacterial and viral diseases and abiotic stresses including drought, salinity, temperature extremes, and nutrient deficiencies (Ahuja et al., 2010; Raza et al., 2020). Biotechnological interventions can be promising solutions to overcome these limitations and to enable sustainable use of Brassicaceae crops. Plant tissue culture techniques such as micropropagation, callus culture, cell suspension culture and hairy root culture offer a way for rapid multiplication of elite genotypes and controlled production of valuable secondary metabolites. These methods allow for year-round production regardless of environmental factors and have been shown to increase the accumulation of bioactive compounds such as glucosinolates, phenolics and flavonoids (Murthy et al., 2014; Bhat & Yadav, 2022).

Great progress has been made in Brassica crop improvement by recent advances in molecular breeding and genetic engineering. Cultivars with better nutritional quality, better stress tolerance and increased resistance to diseases and pests are being developed using marker-assisted selection, genomic selection, transgenic technologies and genome-editing tools such as CRISPR-Cas systems. Several studies have successfully manipulated genes involved in the biosynthesis and regulation of glucosinolates resulting in increased accumulation of health-promoting phytochemicals without impairing the agronomic performance. This knowledge enables precision breeding and development of cultivars with optimised phytochemical profiles and improved resource-use efficiency. Therefore, biotechnological intervention has become a crucial approach to meet the increasing demand for Brassicaceae vegetables with sustainable production, improved nutritional content, effective utilisation of plant biomass, and tolerance to environmental stresses (Cartea & Velasco, 2008; Kumar et al., 2021).

6. Plant Tissue Culture as a Tool for Brassicaceae Improvement

The use of plant tissue culture for mass propagation of plants is well established. The main advantage of this technique is to produce very large numbers of plantlets, which in theory are identical, from a limited amount of parent material (Clare et al., 1974). It multiplies isolated plant cells, tissues, and organs under *in vitro* conditions to regenerate and propagate the entire plants. Growing such plants ensures effective clonal propagation of genetically superior genotypes of economically important plants (Ivan Iliev et al., 2010).

Establishment of a reliable, reproducible and efficient *in vitro* plant regeneration system with cell and tissue culture is essential for biotechnological application of crop improvement. Compared to conventional breeding approaches which are laborious and highly influenced by environmental factors affecting yield and quality of crop, biotechnological intervention becomes a major requirement. Many studies have been carried out on tissue culture-mediated approaches, such as development of protocols for *in vitro* mass

propagation, somaclonal variation, and secondary metabolite production (Mitra, M. et al., 2020). These recent developments in the field of plant tissue culture have made it one of the most dynamic and promising fields in experimental biology (Kumar, P et al., 2016).

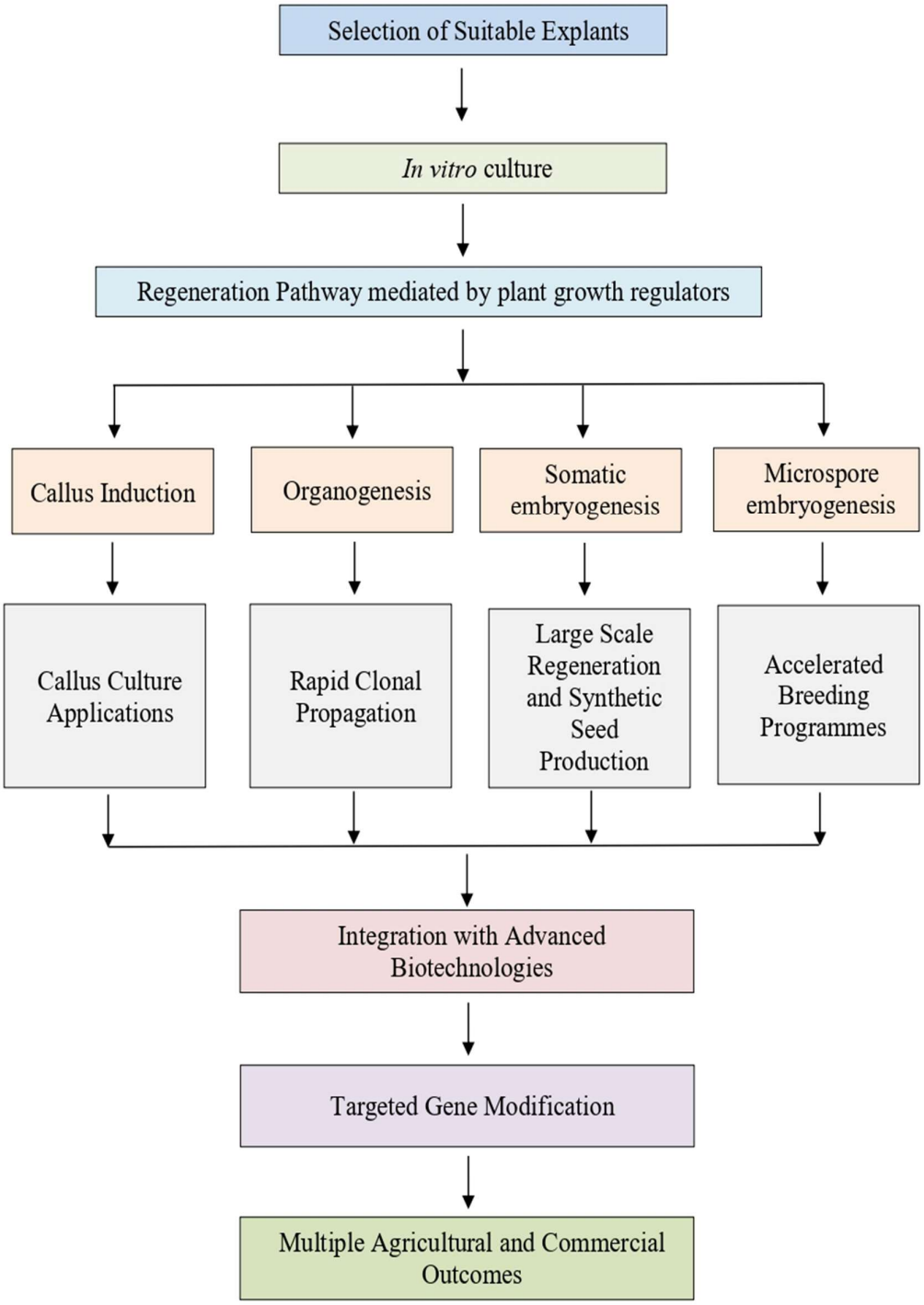
7. *In Vitro* Regeneration Pathways in Brassicaceae

In vitro plant propagation through tissue culture techniques involves two main steps: first, the formation of *in vitro* cell/callus/cell suspension and protoplast culture, and second, either direct or indirect organogenesis or somatic embryogenesis for *in vitro* regeneration. Organogenesis in tissue culture refers to the *in vitro* formation of plant structures such as roots, shoots and leaves. This process can occur either directly from the meristem or indirectly from the callus. To enable plant regeneration through organogenesis, plant growth regulators are modified by adjusting their concentrations in the nutrient medium. This modification influences the formation of callus and the differentiation of adventitious meristems into organs (Ozyigit, I. I. et al., 2023).

Plant growth regulators auxins and cytokinins are both required for *in vitro* cell proliferation and maintenance of cultured plant tissues for extended period. The ratio of these growth regulators high or low determines the morphogenetic pathway of the *in vitro* cultured concentrations result in unorganised growth of a cell mass. This proliferating cell mass is termed as “callus”. It is a disorganised accumulation of parenchyma tissue that contains regions with meristematic potential (Bhojwani, S. S. et al., 2013). Produced from a single differentiated cell; many callus cells are totipotent i.e. able to regenerate the whole plant body (Fehér, A., 2019). It is believed in some cases; process of callus formation involves dedifferentiation of cells. Calli are diverse and are classified into subgroups based on their visible characteristics. The calli that do not show organ regeneration are called friable or compact callus. On the other hand, calli that show varying degrees of organ regeneration are categorised as root, shoot or embryonic callus, depending on the organs they generate (Ikeuchi, M et al., 2013). Callus cultures provide initial stage for single-cell and suspension cultures. They are basis for conducting various studies related to morphogenesis, physiology. Additionally, callus cultures are utilised for production of secondary metabolites. They play a significant role in generating somaclonal variations for crop improvement and practical applications (Bhojwani, S. S. et al., 2013).

Collectively, *in vitro* regeneration systems based on callus induction and organogenesis form the foundation of Brassicaceae biotechnology. These approaches help in rapid multiplication, conservation of elite germplasm, genetic transformation, and production of valuable secondary metabolites in controlled conditions. Also, long term *in vitro* culture and repeated regeneration cycles may result in genetic and epigenetic changes in regenerated plants. Somaclonal variation, has gained quite importance as a source of new variability for crop improvement and has been widely studied in Brassicaceae species for developing desirable agronomic traits.

Fig 1: Brassicaceae Crop Improvement Flowchart



8. Somaclonal Variation: Potential and Limitations

Somaclonal variation is genetic and phenotypic variation among clonally propagated plants. This is useful for developing crops with improved resistance to diseases, pests, or environmental stresses. It requires less

space and time for screening of desirable traits *in vitro* unlike cross seedlings of perennial crops, which require a great deal of land area and time. Although this process can be unpredictable, this variability provides opportunity for rapid regeneration and genetic diversity that include new traits not achievable through conventional breeding methods. In Brassicaceae, somaclonal variation has been explored for stress tolerance, yield traits, and biochemical enhancement (Kaeppler et al., 2000) (Ferreira et al., 2023)

To highlight chronological evolution of key regeneration and biotechnological milestones in Brassicaceae key advancements have been studied and are compiled in following table summarising five decades (1974–2025) of *in-vitro* culture, regeneration, and genome engineering research in Brassicaceae crops, showing a clear evolution of techniques and applications.

Early studies on Brassicaceae in 1970s–1980s were focused on basic regeneration protocols, including callus induction, shoot organogenesis, and protoplast-to-plant systems in cabbage, Brussels sprouts, kohlrabi, and rapeseed. These primary methods established that diverse *Brassica* explants (hypocotyls, cotyledons, leaves, microspores) could regenerate whole plants. From the 1990s to early 2000s, research expanded toward somatic embryogenesis, microspore culture, doubled haploid production, hairy-root systems, and cryopreservation. These advances enabled faster breeding, stable metabolite production, and transformation platforms.

Between 2010 and 2020, emphasis shifted to metabolic studies (e.g., glucosinolates), elicitation, synthetic seeds, and genotype-specific protocol refinement, alongside large-scale DH pipelines integrated with genomic selection.

Recently from 2020, CRISPR/Cas genome editing, protoplast transfection, DNA-free editing, and multiplex targeting in polyploid genomes became central themes.

Table 1. Major Milestones in *In Vitro* Regeneration and Biotechnological Applications in Brassicaceae Species (1974–2025)

No	Common Name	Botanical Name	*In-Vitro* Work (Explant & Method)	Key Result	Year	Reference
1	Brussels sprouts	*Brassica oleracea* var. *gemmifera*	Callus induction & plantlet regeneration from sprout tissue	First regeneration from sprouts; foundational *Brassica*	1974	Clare & Collin

				protocol		
2	Cabbage	*Brassica oleracea* var. *capitata, Brassica napus, and Brassica campestri*	Leaf-disc culture, hypocotyl regeneration	Consistent direct shoots from explants	1981	Dunwell
3	Cabbage	*Brassica oleracea*	Mesophyll protoplast → somatic embryo → plant	Early protoplast-to-plant success	1985	Fu, Yy et al.
4	Kohlrabi	*Brassica oleracea* var. *gongylodes*	Shoot regeneration using high 6-Benzyladenine (BA)	First regeneration in kohlrabi	1988	Glendening & Sjölund
5	Rapeseed	*Brassica napus*	Cryopreservation of microspores	Cryo-stored microspores remained embryogenic	1988	David G. Charne et al.
6	Chinese cabbage	*Brassica rapa*	Protoplast isolation	High yield protoplasts	1989	Yamashita, Y et al.
7	Rapeseed	*Brassica napus*	Hairy-root via *A. rhizogenes* infection	Hairy roots + transformed shoot regeneration	1991	Damgaard et al.
8	Radish	*Raphanus sativus*	hypocotyl segments to callus to somatic embryos	Embryos to plantlets and transplant onto potted	1995	Jeong WJ et al.

				soil		
9	Cauliflower	*Brassica oleracea* var. *botrytis*	Hybrid seed production	Shoot organogenesis standardised	1999	Bhalla et al.
10	Rapeseed	*Brassica napus*	Induction of somatic embryogenesis from hypocotyls and cotyledons	secondary embryos, and regenerated plants	2000	W.L. Koh et al.
11	Broccoli	*Brassica oleracea var. italica*	leaf-explants regenerated to produce clones retaining the transgenes	Transgene maintained	2003	J. Cao et al.
12	Cauliflower	*Brassica oleracea* var. *botrytis*	Direct organogenesis & Plant Growth Regulator (PGR) optimisation; Silver nitrate ($AgNO_3$) additive studies	Use of $AgNO_3$ reduced browning and improved shoot regeneration efficiency	2006	Qin, Y et al.
13	Mustard greens	*Brassica juncea*	Organogenesis from cotyledon node; Agrobacterium-mediated transformation attempts	Efficient shoot induction protocols: moderate transformability reported	2011	Bhuiyan, M. S et al.
14	Rapeseed	*Brassica napus*	Synthetic seed	Encapsulate	2013	Eivazi, A

			production from somatic embryos (alginate encapsulation)	d somatic embryos with decent conversion rates; pilot storage studies		et al.
15	Broccoli, rutabaga, turnip, China rose radish, red radish	*Brassica oleracea L. var. italica, Brassica napus L. var. napobrassica, Brassica rapa L. subsp. Rapa, R. sativus L. cv. China rose, R. sativus L. cv. Rambo*	Use of elicitors (Methyl jasmonate (MeJA), etc.) for glucosinolate output	Clear increase in targeted metabolites under elicitation	2014	Baenas N et al.
16	Cauliflower and Broccoli	*Brassica oleracea var. botrytis and Brassica* oleracea a L. var. italica	Optimisation of rooting using auxins (Indole-3-butyric acid (IBA), α-Naphthaleneacetic acid (NAA)) for higher acclimatisation rates	Optimised rooting regimes increased field survival from *in-vitro* plants	2012 and 2015	Kieffer, M., & Fuller, M. P. and Kumar, P., & Srivastava, D. K
17	Broccoli	*Brassica oleracea* var. *italica*	Optimisation of Thidiazuron (TDZ) & 6-Benzylaminopu	TDZ-based protocols significantly increased	2014 and 2015	Ravanfar, S. A. et al. and Kumar &

			rine (BAP) for direct shoot organogenesis from cotyledon explants	shoot induction in several cultivars		Srivastava , 2015
18	Cabbage	*Brassica oleracea var. capitata*	Comparative regeneration studies across cultivars (explant × PGR × genotype)	Confirmed wide genotype-driven variability; suggested cultivar-specific protocols	2015	Gerszberg , A et al.
19	Kale	*Brassica oleracea* var. *acephala*	Hairy root culture for glucosinolate study	Hairy roots produced targeted glucosinolates and were stable producers in culture	2016	Lee, S. Y et al.
20	Radish	*Raphanus sativus*	leaf tissues, total Ribonucleic acid (RNA) to construct a Complementary DNA (cDNA) library	systematic identification of bolting and flowering-related 21 genes based on transcriptome sequencing and	2016	Nie, S et al.

				assembly in radish		
21	Mustard	*Brassica juncea*	*In-vitro* anther culture and androgenesis studies	Optimised donor plant stage and pretreatments for androgenesis in some genotypes	2016	Dipti, D. et al.
22	Rapeseed	*Brassica napus*	Large-scale DH production pipelines combined with genomic selection	Integration of DH + genomic selection accelerated breeding cycles	2016	Zou, J. et al.
23	Arabidopsis	*Arabidopsis thaliana*	Protocol development for somatic embryogenesis and transformation methodologies used as model to translate to *Brassica*	Model-system protocols informed *Brassica* method improvements	2019	Momoko Ikeuchi et al.
24	Ethiopian mustard	*Brassica carinata*	Somatic embryogenesis from cotyledon and hypocotyl explants	Successful somatic embryogenesis in some cultivars enabling plant	2019	Shah, R. U. et al.

				regeneration and DH approaches		
25	Cabbage	*Brassica oleracea var. capitata*	Comparative genome editing across cultivars — polyploid editing strategies	Demonstrated methods to target multiple homeologs in polyploid genomes	2019	Ma, C. et al.
26	Rapeseed	*Brassica napus*	Protocol for microspore culture	Isolate and culture microspore	2020	Corral-Martínez, P et al.
27	Turnip	*Brassica rapa* ssp. *rapa*	Microspore culture and embryogenesis optimisation	Identified genotypes with high microspore embryogenic response; DHs used for breeding	2020	Shumilina, D. et al.
28	Rapeseed and *B. rapa*	*Brassica napus* and *Brassica rapa* hybrids	Microspore embryogenesis and DH production in interspecific backgrounds	DH production used to stabilise hybrid traits for breeding	2020	Daurova, A et al.
29	Mustard	*Brassica juncea*	Protoplast fusion for trait introgression (somatic hybrids with other crucifers)	Successful somatic hybrids with desirable traits (e.g., stress	2020	Kumari, P et al.

				tolerance)		
30	Watercress	*Nasturtium officinale*	Micropropagation protocols and secondary metabolite profiling *in vitro*	Established micropropagation and metabolite production; *in vitro* plants used for nutraceutical studies	2020	Klimek-Szczykutowicz, M. et al.
31	Rapeseed	*Brassica napus*	Protoplast transfection and transient expression assays	Improved protoplast viability and transient gene expression useful for quick functional tests	2021	Li et al.
32	Chinese cabbage	*Brassica rapa* ssp. *pekinensis*	Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) editing for flowering time / quality traits using Agrobacterium	Edited traits with phenotype validation; challenges remain in editing polyploid copies	2021	Hong, J. K. et al.

			& protoplast approaches			
33	Rapeseed	*Brassica napus*	Protoplast-to-plant pipelines improved for editing (Ribonucleoprotein (RNP) delivery)	Increased efficiency of edited plant recovery with transient edits Deoxyribonucleic acid (DNA)-free)	2022	Li et al.
34	*Brassica* napus	*Brassica napus*	CRISPR multiplex editing to modify oil/fatty acid composition (Fatty Acid Desaturase 2 (FAD2), Fatty Acid Elongation 1 (FAE1)	Successfully altered seed oil fatty acid profiles with minimal off-targets in certain cultivars	2022	Shi, J et al.
35	Broccoli	*Brassica oleracea*	Using CRISPR, edit *Brassica oleracea* MYB Domain Protein 28 (BolMYB28) in broccoli	aiming to increase glucoraphanin (a Glucosinolate (GSL))	2022	Kim, YC. et al.
36	Rapeseed	*Brassica napus*	Hairy root	Hairy roots	2022	Jedličková

			transformants used as platforms for CRISPR tests and metabolic screens	used as quick functional platforms before whole-plant work		, V., et al.
37	Rocket / Arugula	*Eruca sativa*	*In vitro* shoot multiplication and phytochemical profiling	Micropropagation successful; *in-vitro* shoots contain glucosinolate	2023	Banjac, N et al.
38	Kohlrabi	*Brassica oleracea* var. *gongylodes*	Shoot multiplication & micropropagation refinement	Established efficient multiplication protocols for commercial propagation	2023	Ćosić, T. et al.
39	Rapeseed	*Brassica napus*	Microspore culture–based DH production in *Brassica napus*.	Improved efficiency and understanding of DH production and its use for studying agronomically important traits.	2023	Starosta, E. et al.
40	*Brassica*	*Brassica*	CRISPR/Cas	Improved	2025	Ton, L. B.

	species	species	genome editing using *Brassica* explants via tissue culture.	resistance to pathogens and tolerance to abiotic stresses.		et al.

The studies carried out collectively summarised that somaclonal variation generated through *in vitro* culture systems plays a significant role in the improvement of Brassicaceae crops.

9. Emerging Biotechnological Approaches in Brassicaceae and Future Prospects

Recent rapid advancements in biotechnological tools complement conventional *in vitro* regeneration systems in Brassicaceae. The regeneration systems provide year-round propagation and quick multiplication of superior genotypes that may contribute to sustainable agriculture and meet the increasing demand for functional foods. Application of elicitors in *in vitro* cultures enhances secondary metabolite production. Genome editing technologies have emerged as efficient tools for precise genetic modification. Successful editing of genes associated with flowering time, oil composition, glucosinolate biosynthesis, and stress tolerance has been reported in species such as *Brassica napus*, *Brassica rapa*, and *Brassica oleracea*. These innovations expand the potential of Brassicaceae crops for agricultural, nutritional, and pharmaceutical applications. These techniques will further aid in translating laboratory-scale discoveries into commercial agricultural applications. With continued refinement and integration of biotechnological tools, Brassicaceae crops are poised to contribute substantially to sustainable agriculture, food security, and human health (Raza et al., 2020) (Li, J et al.,2022).

10. Conclusion

Plant tissue culture and its utilisation give striking opportunities for mass propagation, secondary metabolite production and genetic improvement within the Brassicaceae. In conclusion, these advances have significantly improved regeneration from classical techniques to modern tools. As we continue to explore the potential of this versatile family, Brassicaceae will play a significant role in addressing global challenges in nutrition, agriculture and human health. This review directs and promotes the existing knowledge of nutrient status of Brussels sprouts, Chinese cabbage and other Brassicaceae plants which will be beneficial if grown by cultivators for commercial gain.

Declarations

- **Funding**

Not Applicable

- **Conflict of interest**

Not Applicable

Acknowledgements:

We are grateful for the necessary facilities provided by the Plant Biotechnology Research Lab, Ramniranjan Jhunjhunwala College (Empowered Autonomous) throughout this study.

References

Ahuja, I., de Vos, R. C. H., Bones, A. M., & Hall, R. D. (2010). Plant molecular stress responses face climate change. *Trends in Plant Science*, 15(12), 664–674.

Al-Shehbaz, I. A. (2011). Brassicaceae (mustard family). *eLS*.

Al-Shehbaz, I. A., Beilstein, M. A., & Kellogg, E. A. (2006). Systematics and phylogeny of the Brassicaceae (*Cruciferae*): an overview. *Plant systematics and evolution*, *259*, 89-120.

Avato, P., & Argentieri, M. P. (2015). Brassicaceae: A rich source of health improving phytochemicals. *Phytochemistry reviews*, *14*(6), 1019-1033.

Baenas N, García-Viguera C, Moreno DA. (2014). Biotic elicitors effectively increase the glucosinolates content in Brassicaceae sprouts. J Agric Food Chem. 2014 Feb 26;62(8):1881-9.

Banjac, N., Krstić-Milošević, D., Mijalković, T., Petrović, M., Ćosić, T., Stanišić, M., & Vinterhalter, B. (2023). *In vitro* shoot multiplication and regeneration of the recalcitrant rocket (Eruca sativa Mill.) variety Domaća Rukola. *Horticulturae*, *9*(5), 533.

Bhalla, P.L., de Weerd, N. *In vitro* propagation of cauliflower, *Brassica oleracea var. botrytis* for hybrid seed production. *Plant Cell, Tissue and Organ Culture* 56, 89–95 (1999).

Bhat, S. R., & Yadav, A. K. (2022). Biotechnological approaches for genetic improvement of Brassica crops. *Plant Cell, Tissue and Organ Culture*, 148, 1–20.

Bhojwani, S. S., & Dantu, P. K. (2013). *Plant tissue culture: an introductory text* (Vol. 318). India: Springer.

Bhuiyan, M. S. U., Min, S. R., Jeong, W. J., Sultana, S., Choi, K. S., Lim, Y. P., ... & Liu, J. R. (2011). An improved method for Agrobacterium-mediated genetic transformation from cotyledon explants of Brassica juncea. Plant Biotechnology, 28(1), 17-23.

Cao, J., Earle, E.D. (2003) Transgene expression in broccoli (*Brassica oleracea* var. *italica*) clones propagated *in vitro* via leaf explants. *Plant Cell Rep* **21**, 789–796.

Cartea, M. E., & Velasco, P. (2008). Glucosinolates in Brassica foods: Bioavailability in food and significance for human health. *Phytochemistry Reviews*, 7, 213–229.

Cartea, M. E., Francisco, M., Soengas, P., & Velasco, P. (2010). Phenolic compounds in Brassica vegetables. *Molecules*, *16*(1), 251-280.

Charne DG, Pukacki P, Kott LS, Beversdorf WD. (1988). Embryogenesis following cryopreservation in isolated microspores of rapeseed (*Brassica napus L.*). Plant Cell Rep. 1988 Oct;7(6):407-9.

Clare, M. V., & Collin, H. A. (1974). The production of plantlets from tissue cultures of Brussels Sprout (*Brassica oleracea L. var gemmifera* DC). *Annals of Botany*, *38*(5), 1067-1076.

Corral-Martínez, P., Camacho-Fernández, C., Seguí-Simarro, J.M. (2020). Isolated Microspore Culture in *Brassica napus*. In: Bayer, M. (eds) Plant Embryogenesis. Methods in Molecular Biology, vol 2122. Humana, New York, NY.

Ćosić, T., Raspor, M., Motyka, V., Cingel, A., & Ninković, S. (2023). *In vitro* growth and regeneration of *brassica oleracea var. gongylodes*: a decade of research. *Horticulturae*, *9*(6), 674.

Damgaard, O., Rasmussen, O. (1991) Direct regeneration of transformed shoots in *Brassica napus* from hypocotyl infections with *Agrobacterium rhizogenes*. *Plant Mol Biol* **17**, 1–8.

Daurova, A., Daurov, D., Volkov, D., Zhapar, K., Raimbek, D., Shamekova, M., & Zhambakin, K. (2020). Doubled haploids of interspecific hybrids between *Brassica napus* and *Brassica rapa* for canola production with valuable breeding traits. *Ocl*, *27*, 45.

Dipti, D., Veena Chawla, V. C., Yadav, N. R., Yadav, R. C., & Priyanka, P. (2016). Comparative response of different genotypes of *Brassica Juncea* to anther culture.

Eivazi, A., & Mousavi-Anzabi, S. H. (2013). *In vitro* plant regeneration from alginate-encapsulated somatic embryos of rapeseed (*Brassica napus cv. Tallayeh*). International Journal of Traditional and Herbal Medicine. Vol., 1 (1), 13-18.

Fahey JW, Zalcmann AT, Talalay P (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56:5–51

Fahey, J. W. (2015). *Brassica*: characteristics and properties. In *Encyclopedia of food and health* (pp. 469-477). Elsevier Inc.

Fehér, A. (2019). Callus, dedifferentiation, totipotency, somatic embryogenesis: what these terms mean in the era of molecular plant biology? *Frontiers in plant science*, *10*, 536.

Ferreira, M. D. S., Rocha, A. D. J., Nascimento, F. D. S., Oliveira, W. D. D. S., Soares, J. M. D. S., Rebouças, T. A., ... & Amorim, E. P. (2023). The role of somaclonal variation in plant genetic improvement: A systematic review. *Agronomy*, *13*(3), 730.

Fu, Yy., Jia, Sr. & Lin, Y. (1985) Plant regeneration from mesophyll protoplast culture of cabbage (*Brassica oleracea* var 'capitata'). *Theoret. Appl. Genetics* 71, 495–499.

Gerszberg, A., Hnatuszko-Konka, K. & Kowalczyk, T. (2015) *In vitro* regeneration of eight cultivars of *Brassica oleracea* var. *capitata*. *In Vitro Cell.Dev.Biol.-Plant* 51, 80–87.

Glendening, T. M., & Sjolund, R. (1988). *In vitro* propagation of kohlrabi from leaf explants. *HortScience*, *23*(4), 772-772.

Hong, J. K., Suh, E. J., Park, S. R., Park, J., & Lee, Y. H. (2021). Multiplex CRISPR/Cas9 mutagenesis of BrVRN1 delays flowering time in Chinese cabbage (*Brassica rapa L. ssp. pekinensis*). *Agriculture*, *11*(12), 1286.

Ikeuchi, M., Favero, D. S., Sakamoto, Y., Iwase, A., Coleman, D., Rymen, B., & Sugimoto, K. (2019). Molecular mechanisms of plant regeneration. *Annual review of plant biology*, *70*(1), 377-406.

Ikeuchi, M., Sugimoto, K., & Iwase, A. (2013). Plant callus: mechanisms of induction and repression. *The plant cell*, *25*(9), 3159-3173.

Iliev, I., Gajdošová, A., Libiaková, G., & Jain, S. M. (2010). Plant micropropagation. *Plant cell culture: essential methods*, *1*, 1-23.

J. M. DUNWELL, *In vitro* Regeneration from Excised Leaf Discs of Three *Brassica* Species, *Journal of Experimental Botany*, Volume 32, Issue 4, August 1981, Pages 789–799.

Jedličková, V., Mácová, K., Štefková, M., Butula, J., Staveníková, J., Sedláček, M., & Robert, H. S. (2022). Hairy root transformation system as a tool for CRISPR/Cas9-directed genome editing in oilseed rape (*Brassica napus*). *Frontiers in plant science*, *13*, 919290.

Jeong WJ, Min SR, Liu JR. (1995) Somatic embryogenesis and plant regeneration in tissue cultures of radish (Raphanus sativus L.). Plant Cell Rep. 1995 Jul;14(10):648-51.

Jong-In Park, Nasar Uddin Ahmed, Hye-Ran Kim, Ill-Sup Nou. (2012). Advances in *in vitro* Culture of the Brassicaceae Crop Plants. *Journal of Plant Biotechnology*, *39*(1), 13-22.

Kaeppler, S. M., Kaeppler, H. F., & Rhee, Y. (2000). Epigenetic aspects of somaclonal variation in plants. *Plant molecular biology*, *43*(2), 179-188.

Kieffer, M., & Fuller, M. P. (2012). *In vitro* propagation of cauliflower using curd microexplants. In *Protocols for Micropropagation of Selected Economically Important Horticultural Plants* (pp. 329-339). Totowa, NJ: Humana Press.

Kim, YC., Ahn, W.S., Cha, A. *et al.* (2022) Development of glucoraphanin-rich broccoli (*Brassica oleracea* var. *italica*) by CRISPR/Cas9-mediated DNA-free *BolMYB28* editing. *Plant Biotechnol Rep* **16**, 123–132.

Klimek-Szczykutowicz, M., Dziurka, M., Blažević, I., Đulović, A., Granica, S., Korona-Glowniak, I., ... & Szopa, A. (2020). Phytochemical and biological activity studies on Nasturtium officinale (watercress) microshoot cultures grown in RITA® temporary immersion systems. *Molecules*, *25*(22), 5257.

Koh WL, Loh CS. (2000) Direct somatic embryogenesis, plant regeneration and *in vitro* flowering in rapid-cycling *Brassica napus*. Plant Cell Rep. 2000 Dec;19(12):1177-1183.

Kumar, P., & Srivastava, D. K. (2015). High frequency organogenesis in hypocotyl, cotyledon, leaf and petiole explants of broccoli (*Brassica olerace a L. var. italica*), an important vegetable crop. *Physiology and Molecular Biology of Plants*, *21*(2), 279-285.

Kumar, P., & Srivastava, D. K. (2016). Biotechnological applications in *in vitro* plant regeneration studies of broccoli (*Brassica oleracea L. var. italica*), an important vegetable crop. *Biotechnology letters*, *38*(4), 561-571.

Kumar, P., Srivastava, D.K. (2015). Effect of potent cytokinin thidiazuron on *in vitro* morphogenic potential of broccoli (*Brassica oleracea* L. var. *italica*), an important vegetable crop. *Ind J Plant Physiol.* **20**, 317–323.

Kumar, S., Yadava, S. K., Chauhan, N. S., & Kumar, P. (2021). CRISPR/Cas-mediated genome editing in Brassica crops: Applications and future prospects. *Frontiers in Plant Science*, 12, 682459.

Kumari, P., Singh, K. P., Kumar, S., & Yadava, D. K. (2020). Development of a yellow-seeded stable allohexaploid brassica through inter-generic somatic hybridization with a high degree of fertility and resistance to Sclerotinia sclerotiorum. *Frontiers in Plant Science*, *11*, 575591.

Lee, S. Y., Bong, S. J., Kim, J. K., & Park, S. U. (2016). Glucosinolate biosynthesis as influenced by growth media and auxin in hairy root cultures of kale (*Brassica oleracea var. acephala*). *Emirates Journal of Food and Agriculture*, *28*(4), 277.

Li, J., Yu, X., Zhang, C., Li, N., & Zhao, J. (2022). The application of CRISPR/Cas technologies to Brassica crops: current progress and future perspectives. *Abiotech*, *3*(2), 146-161.

Li, X., Sandgrind, S., Moss, O., Guan, R., Ivarson, E., Wang, E. S., ... & Zhu, L. H. (2021). Efficient protoplast regeneration protocol and CRISPR/Cas9-mediated editing of glucosinolate transporter (GTR) genes in rapeseed (*Brassica napus L.*). *Frontiers in Plant Science*, *12*, 680859.

Ma, C., Zhu, C., Zheng, M., Liu, M., Zhang, D., Liu, B., ... & Song, H. (2019). CRISPR/Cas9-mediated multiple gene editing in *Brassica oleracea var. capitata* using the endogenous tRNA-processing system. *Horticulture Research*, *6*.

Mitra, M., & Gantait, S. (2020). Tissue Culture-Mediated Biotechnological Advancements in Genus Brassica. *Brassica Improvement: Molecular, Genetics and Genomic Perspectives*, 85-107.

Murthy, H. N., Lee, E. J., & Paek, K. Y. (2014). Production of secondary metabolites from cell and organ cultures: Strategies and approaches for biomass improvement and metabolite accumulation. *Plant Cell, Tissue and Organ Culture*, 118, 1–16.

Nie, S., Li, C., Xu, L., Wang, Y., Huang, D., Muleke, E. M., ... & Liu, L. (2016). De novo transcriptome analysis in radish (*Raphanus sativus L.*) and identification of critical genes involved in bolting and flowering. *BMC genomics*, *17*(1), 389.

Ozyigit, I.I.; Dogan, I.; Hocaoglu-Ozyigit, A.; Yalcin, B.; Erdogan, A.; Yalcin, I.E.; Cabi, E.; Kaya, Y. (2023) Production of Secondary Metabolites Using Tissue Culture-Based Biotechnological Applications. *Front. Plant Sci.* 2023, *14*, 1132555.

Podsędek, A. (2007). Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. *LWT-Food science and Technology*, *40*(1), 1-11.

Qin, Y., GAO, L. H., Pulli, S., & GUO, Y. D. (2006). Shoot differentiation, regeneration of cauliflower and analysis of somaclonal variation by RAPD. *Hereditas*, *143*(2006), 91-98.

Ravanfar, S. A., Aziz, M. A., Rashid, A. A., & Salim, S. H. A. H. I. D. A. (2014). *In vitro* adventitious shoot regeneration from cotyledon explant of *Brassica oleracea subsp. italica* and *Brassica oleracea subsp. capitata* using TDZ and NAA. *Pak J Bot*, *46*(1), 329-335.

Raza, A., Hafeez, M. B., Zahra, N., Shaukat, K., Umbreen, S., Tabassum, J. & Hasanuzzaman, M. (2020). The plant family Brassicaceae: Introduction, biology, and importance. *The Plant Family Brassicaceae: Biology and Physiological Responses to Environmental Stresses*, 1-43.

Shah, R. U., & Munir, I. (2019). Efficient *in vitro* plant regeneration through somatic embryogenesis from callus induction method for *Brassica carinata*. *Sarhad J. Agric*, *35*, 314-319.

Sharma, P., & Kapoor, S. (2015). Biopharmaceutical aspects of Brassica vegetables. *Journal of Pharmacognosy and Phytochemistry*, *4*(1), 140-147.

Shi, J., Ni, X., Huang, J., Fu, Y., Wang, T., Yu, H., & Zhang, Y. (2022). CRISPR/Cas9-Mediated Gene Editing of *BnFAD2* and *BnFAE1* Modifies Fatty Acid Profiles in *Brassica napus*. *Genes*, *13*(10), 1681.

Shumilina, D., Kornyukhin, D., Domblides, E., Soldatenko, A., & Artemyeva, A. (2020). Effects of genotype and culture conditions on microspore embryogenesis and plant regeneration in *Brassica Rapa ssp. Rapa L. Plants*, *9*(2), 278.

Sjödin, C. (1992). Brassicaceae, a plant family well suited for modern biotechnology. *Acta Agriculturae Scandinavica B-Plant Soil Sciences*, *42*(4), 197-207.

Starosta, E., Szwarc, J., Niemann, J., Szewczyk, K., & Weigt, D. (2023). *Brassica napus* haploid and double haploid production and its latest applications. *Current Issues in Molecular Biology*, *45*(5), 4431-4450.

Ton, L. B., Qayyum, Z., Amas, J., Thomas, W. J., Edwards, D., Batley, J., & Dolatabadian, A. (2025). Applications of CRISPR/Cas tools in improving stress tolerance in Brassica crops. *Frontiers in Plant Science*, *16*, 1616526.

Yamashita, Y., Shimamoto, K. (1989). Regeneration of Plants from Cabbage (*Brassica oleracea* var. *capitata*) Protoplasts. In: Bajaj, Y.P.S. (eds) Plant Protoplasts and Genetic Engineering I. Biotechnology in Agriculture and Forestry, vol 8. Springer, Berlin, Heidelberg.

Zou, J., Zhao, Y., Liu, P., Shi, L., Wang, X., Wang, M., ... & Reif, J. C. (2016). Seed quality traits can be predicted with high accuracy in Brassica napus using genomic data. *PLoS One*, *11*(11), e0166624.