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Review Article

Trends on conventional and modern breeding techniques of *Brassica* vegetables

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Abstract

Vegetable crops, particularly those of the cole variety, serve as pivotal entities in the exploitation of heterosis to engineer high-yielding hybrids. Despite their inherent significance, challenges such as labor intensity and a dearth of skilled personnel hinder large-scale hybrid seed production endeavors. Cole crops, belonging to the Brassicaceae family, exhibit discernible heterotic responses, displaying attributes such as augmented yield, uniform maturation, precocious flowering, and robustness against diverse stressors. Employing controlled pollination techniques, notably self-incompatibility mechanisms and genetic emasculation methods, stands as the cornerstone of economically viable commercial hybrid seed production practices. This comprehensive review delves into the intricate genetic underpinnings governing mechanisms such as genic male sterility, cytoplasmic male sterility, self-incompatibility, and the application of chemical hybridizing agents in cole crops. A critical appraisal of the merits and demerits of these strategies ensues, with a particular focus on cost-effective modalities for large-scale F_1 seed generation. Prominent examples include hybrids such as Pusa Cabbage Hybrid 1, Pusa Cabbage Hybrid 81, and Pusa Snowball Hybrid 1 and 2, cultivated by leading public sector entities and private seed enterprises. Moreover, this review explores advanced biotechnological methodologies, including tissue culture, somatic hybridization, molecular-assisted breeding, genetic engineering, RNAi, and genome editing, aimed at augmenting crucifer improvement programs.

Keywords : *Biotechnological advancements, cole crops, genetic mechanisms, heterosis, self-incompatibility*

Introduction

The term "cole crops" finds its origin in the Latin word "caulis," which translates to "stem" or "stalk" of a plant. In contemporary usage, cole crops specifically refer to the stem brassicas. These plants belong to the Cruciferae family, also known as the mustard family. The Brassicaceae family derives its name from the genus Brassica, which is preeminent among 372 genera in the family and encompasses 3700 distinct species (Gomez-Campo, 1980). All cultivated crops belong to the species *Brassica oleracea*. Cole crops are extensively cultivated across the globe, spanning tropical, sub-tropical, and temperate regions. India stands as the foremost vegetable producer globally, covering an area of 11.24 million hectares with a production of 212.24 million metric tons (NHB 2022-23), ranking second after China. India contributes 12.3% to the global production of cabbage, cauliflower and other cole crops.

Cole vegetables like cabbage, broccoli, Brussels sprouts, kale and cauliflower are esteemed for their nutraceutical properties, gaining traction among consumers and dominating the market. They serve as rich sources of vitamins A and C, dietary fiber, calcium, potassium. These crops boast an array of health benefits attributed to their rich reservoirs of sulphur-containing glucosides and bioactive compounds known as glucosinolates, renowned for their anticancer properties. Broccoli, in particular, stands out for its high levels of vitamin C, soluble fiber, and potent anticancer agents like di-indolylmethane and selenium. With advancements in molecular tools, breeding for nutrient-dense varieties has accelerated, promising enhanced traits like increased sulforaphane content, ultimately mitigating disease risks such as prostate cancer, (Kalia & Singh, 2023).

The confluence of limited arable land, burgeoning population, and escalating land pressures due to urbanization and industrialization has underscored the imperative of boosting productivity. Enhanced productivity in vegetables can be attained through the adoption of F_1 hybrids coupled with improved traits and the standardization of agricultural techniques. However, the relatively higher cost of hybrid seeds stands out as a significant impediment hindering widespread adoption of hybrid vegetable technology. Employing genetic mechanisms such as male sterility, self-incompatibility, and biotechnological tools holds promise in mitigating the expenses associated with hybrid seed production.

1. Conventional breeding methods

These methods rely on the natural inheritance of genetic material from both parental lines. Through successive generations, breeders meticulously select individuals displaying superior phenotypic traits, thereby perpetuating desirable characteristics while gradually eliminating undesirable genetic variants.

Significance of embracing hybrid seed technology

- Early Maturation: Accelerated growth timelines enable quicker harvests.
- Enhanced Yield: Increased crop output ensures greater agricultural productivity.
- Adaptability to Variable Conditions: Improved resilience to diverse environmental factors.
- Disease and Pest Resistance: Heightened tolerance to pathogens and pests fortifies plant health.
- Consistent Quality: Uniformity in produce quality enhances market acceptance.
- Market Suitability: Better adaptability to market demands and preferences.
- Expedited Growth: Streamlined cultivation processes facilitate faster crop development.
- Prolonged Shelf Life: Extended durability compared to heirloom varieties preserves product freshness.

1.1 Male sterility in vegetable crops

Manifestation of male sterility

- Male sterility in plants denotes the incapacity to produce or release viable pollen, stemming from the inadequacy in the formation or maturation of functional stamens, microspores, or gametes.
- It does not involve abnormalities in other male reproductive organs.
- Male sterility results from the inability to develop normal microsporogenesis tissue in the anther.
- Irregularities in pollen maturation contribute to the condition.
- Although the anther remains non-dehiscent, viable pollen is produced, controlled saprophytically.

Classification of male sterility

Among the various types of male sterility, inherited male sterility encompasses two phenotypic-based categories: sporogenous male sterility, structural (or positional) sterility, and functional male sterility. Additionally, genetic-based male sterility includes genetic male sterility, cytoplasmic male sterility, and cytoplasmic genic male sterility. On the other hand, non-inherited genic male sterility comprises chemical male sterility and physiological male sterility.

1.1.1 Genetic male sterility

Pollen formation failure can be attributed to one or multiple nuclear genes, whether dominant or recessive. The majority of naturally occurring or induced male sterility tends to exhibit a recessive genetic nature, with

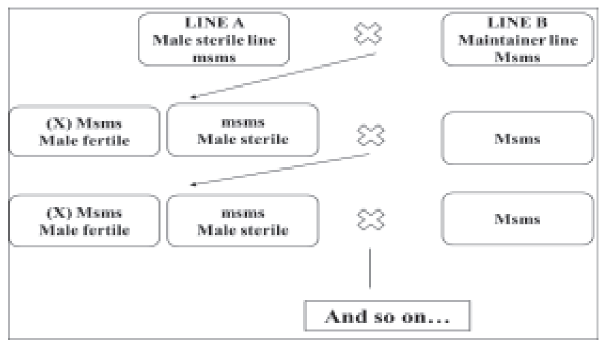

Fig.1: Maintenance of male sterility

exceptions noted in cabbage, broccoli, and genetically modified male sterile varieties.

Development and utilization of GMS

The monogenic recessive gene governing male sterility is transferred into the desired recessive genotype through a backcrossing program. Following the identification of a recessive male sterile mutant plant, the initial step involves crossing it with a plant of the same variety. In

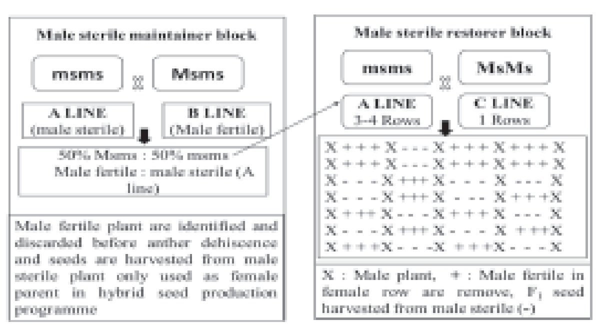

Fig. 2:General scheme for hybrid seed production utilizing GMS

subsequent generations such as the F2, male sterile plants are pollinated with bulk pollen from all the male fertile segregants, and only seeds from male sterile plants are collected to advance to the next generation. Consequently, after four generations, homozygous male fertile (msms) plants are eliminated from the population, leaving behind only heterozygous male fertile (msms) and male sterile (msms) plants. Subsequently, male sterile plants (msms) are sustained by backcrossing them with the heterozygous isogenic line (msms) for male sterility.

In hybrid seed production fields, it is essential to identify and eliminate 50% of male fertile segregants (msms) before they reach the pollen-shedding stage. In certain genetic male sterile (GMS) lines, the ms genes are closely linked with recessive phenotypic marker genes. These marker genes, particularly those expressed during the seedling stage, offer a promising avenue for identifying sterile or fertile plants at an early stage. Seeds are harvested solely from male sterile plants after crossing them with pollen from the male fertile maintainer line.

1.1.2 Transgenic male sterility system (Barnase-Barstar system)

Genetic engineering techniques enable the selective disruption or interference with the normal development of anthers or pollen, leading to male sterility. Although engineered male sterility systems, aside from the barnase-barstar system, are not currently commercially deployed, they hold substantial promise for future hybrid-breeding programs. In this method, male sterile transgenic plants are developed by integrating gene sequences active exclusively in male reproductive organs, thus disrupting pollen or anther development (microsporogenesis). This yields exclusively female (male-sterile) plants suitable for cultivation and hybrid seed production.

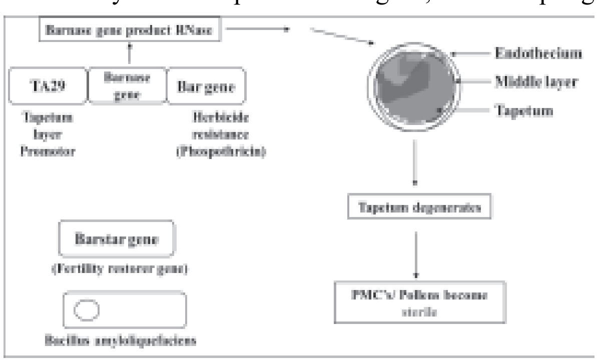

Fig. 3 : Principle of Barstar-Barnase system

The pioneering transgenic male sterility system, known as the Barnase-Barstar system, was developed by Mariani and colleagues in rapeseed and tobacco (Mariani *et al*., 1990; 1992). Transformed plants were generated using a chimeric RNAase gene (Barnase) under the control of a tapetum-specific promoter (TA29). The expression of Barnase (a cytotoxic protein) in the tapetum cells of transformed plants led to the inhibition of tapetum cell and pollen development, resulting in male sterile transgenic plants (Mariani *et al.,* 1990). Crossing these transgenic male sterile plants (hemizygous for Bernese) with normal plants yields 50% hemizygous (Barnase) male sterile F_1 progeny.

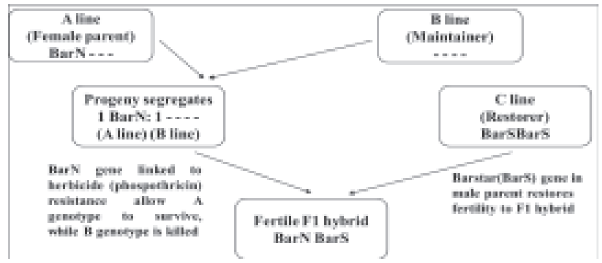

Fig. 4 : Utilization of barnase- barstar system in hybrid seed development

To address the maintenance issue, a transgenic construct incorporating both the Barnase gene and a herbicide-resistant gene has been developed. In this construct, the male sterility gene (Barnase) is linked with the herbicide-resistant gene. Consequently, hemizygous male sterile plants can be crossed with their normal sister plants, and the progeny can be subjected to herbicide spraying. This method ensures the survival of 50% sterile segregants while eliminating 50% fertile segregants through herbicide application. Only the male sterile plants containing Barnase are utilized for crossing with the male fertile line containing the Barstar gene. This restores fertility in the resulting F_1 hybrid, which is then utilized for hybrid seed production.

1.1.3 Environment-sensitive genetic male sterility (EGMS)

Certain genetic male sterile lines exhibit conditional mutations, wherein male sterility manifests only under specific environmental conditions. In the absence of these conditions, the male sterile plants revert to a male fertile state. These conditional mutants are categorized based on the critical environmental factor responsible for the expression of sterility or fertility. For instance, if temperature or photoperiod plays a pivotal role, the lines are designated as Temperature-sensitive Genic Male Sterile (TGMS) or Photoperiod-sensitive Genic Male Sterile (PGMS) lines, respectively. Temperature-sensitive Genic Male Sterile (TGMS) lines, and to a lesser extent, Photoperiod-sensitive Genic Male Sterile (PGMS) lines, have been identified in various vegetable crops such as

Table 1: Environmental genic male sterility (EGMS); Two line hybrid breeding (TGMS–Thermosensitive genic male sterility, PGMS–Photoperiod sensitive genic male sterility)

Vegetable	Mutant	Temperature	Reference
Cabbage	TGMS, PGMS	<10°C	Rundfeldt 1961
Brussels sprouts	TGMS	<10°C	Nieuwhof 1968
Broccoli	TGMS, PGMS	10°-11°C	Rick 1948; Sawhney1983

cabbage, Brussels sprout, broccoli, peppers (both chili and sweet pepper), tomato, and carrot. Notably, many of these lines were initially classified as normal genic male sterile lines (Kumar *et al.,* 2000).

1.1.4 Chemical hybridizing agents (CHA)

Various terms have been employed to describe chemicals capable of inducing male sterility in plants, including gametocide or selective gametocide, pollen suppression, male sterilant, selective male sterilant, pollen suppressant, pollenocide, and androcide. However, considering the ultimate objective of utilizing these chemicals to produce hybrids, the term "chemical hybridizing agent" (CHA) is preferred (McRae, 1985). The potential of certain chemicals, such as maleic hydrazide, to induce selective male sterility was initially demonstrated in maize during the 1950s (Moore, 1950; Naylor, 1950).

Table 2: Chemical hybridizing agents (CHA) for inducing male sterility in cole crops

Vegetable	Applied chemicals	Remarks	Reference
Cole crops	GA3	Reported promising For utilization	Van der Meer &Van Dam, 1979

1.1.5 Cytoplasmic male sterility (CMS)

Cytoplasmic factor-mediated male sterility, known as cytoplasmic male sterility (CMS), is observed in several plant species including carrot, sweet pepper, radish, turnip, cauliflower, cabbage, broccoli, Chinese cabbage, and cucumber. This trait is inherited maternally, as the mitochondrial genome (mt-genome) governs the expression of male sterility, and mitochondria are typically excluded from the pollen during fertilization.CMS can be efficiently transferred to a particular strain by utilizing that strain as the pollinator (referred to as the recurrent parent) in successive generations of a backcrossing program. Following 6-7 backcrosses, the nuclear genotype of the male sterile line becomes nearly identical to that of the recurrent pollinator strain.

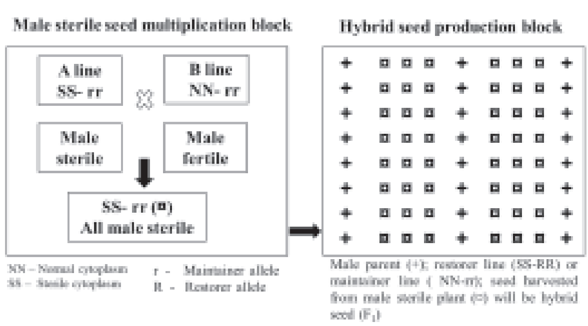

Fig. 5:Utilization of CMS line in hybrid seed production

Utilization of CMS

Cytoplasmic male sterility (CMS) can be sustained by crossing a male sterile line (referred to as the A line) with the pollinator strain (known as the maintainer line), which is utilized as a recurrent parent in the backcrossing program. This approach ensures the preservation of CMS because the nuclear genotype of the maintainer line is identical to that of the newly developed male sterile line.

Ogura cytoplasm

Cytoplasmic male sterility (CMS) was initially identified in a cultivar of Japanese radish by Ogura (1968). This sterility cytoplasm was subsequently introduced into the *Brassica oleracea* genome through repeated backcrosses with broccoli, as reported by Bannerot *et al.* (1974). However, the CMS observed in cabbage had the drawback of chlorosis at low temperatures (below 12°C), which was later addressed by replacing it with protoplast fusion (combining radish chlorosis with *Brassica oleracea*), as described by Robertson *et al.* (1987). This modification resulted in a significant increase in curd yield, ranging from 40% to 75% compared to the self-incompatibility (SI) system, across different maturity groups, as reported by Kalia 2008 and Dey *et al.* (2011).

Recently, CMS lines based on the Ogura system have been developed in snowball cauliflower, namely Ogu1A, Ogu2A, and Ogu3A, for hybrid development in cauliflower. In India, the IARI regional station at Katrain has developed two cabbage hybrids, H-64 and KCH-4, utilizing cytoplasmic male sterility. The 'Anand' cytoplasm, originating from the wild species *Brassica tournefortii,* was transferred from *Brassica rapa to Brassica oleraceae* through a cybridization process. The presence of 'Anand' chloroplasts with a *Brassica oleracea* nucleus did not result in cold temperature chlorosis, unlike in 'Ogura' CMS plants, as observed by Cardi and Earle (1997).

1.2 Self-incompatibility (SI)

Self-incompatibility is a genetic mechanism in plants that prevents them from setting seeds upon self-pollination, even when both male and female gametes are viable. Lewis (1954) proposed various classifications of self-incompatibility, primarily delineating two main types:

- Heteromorphic system
- Pin type
- Thrum type
- Homomorphic system
- Sporophytic self-incompatibility (found in Cole crops)
- Gametophytic self-incompatibility

1.2.1 Sporophytic self-incompatibility

Sporophytic self-incompatibility (SSI) was initially observed in radish by Stout in 1920. The inheritance pattern of SSI was first demonstrated by Bateman in 1955. He described the control of self-incompatibility (SI) in the Brassicaceae family by a single Mendelian locus, known as the S (Sterility) locus, which exhibits multiple alleles. The number of S alleles at the S-locus has been reported to be 34 in Raphanus sativus and 60 in *Brassica oleracea*.

Molecular mechanism of SSI in Brassicaceae

- SRK (S-locus receptor kinase); (Stein *et al*., 1991)
- SP11(S-locus protein 11)/SCR (S-locus cysteine rich); (Suzuki *et al*., 1999; Takayama *et al*., 2000)
- SLG (S-locus glycoproteins)

The male and female S determinant genes, namely SP11 and SRK, are situated at the S-locus. SP11 is primarily expressed in the tapetum cells of anther locules and undergoes accumulation on the pollen surface as pollen matures. In the process of self-pollination, SP11 molecules penetrate the papilla cell wall and engage in S-allele specific interactions with SRK. Upon phosphorylation, SRK interacts with MLPK. The subsequent signal transduction pathway leading to the rejection of self-pollen remains to be fully elucidated.

Assessment of SI

- The initial method devised to quantify self-incompatibility (SI) relied on assessing the seed set following each particular self- or cross-pollination event.
- More recently, fluorescent microscopic observations have offered a more dependable means of directly measuring the compatibility or incompatibility reaction within a shorter timeframe, typically within 12-15 hours post-pollination.

$$\text{Fertility index (FI)} = \frac{\text{Average no. of seeds per siliqua from bud pollination}}{\text{Average no. of seeds per siliqua from self-pollination in freshly opened flowers}}$$

If a line having fertility index-

- >2 SI line
- <1 SC line
- 1-2 Pseudo-SI

Maintenance of SI line

This critical procedure involves the forced selfing of self-incompatible plants, which can be achieved through various methods such as bud pollination or treatment with CO_2 gas (CO_2 enrichment) or sodium chloride. These approaches effectively disrupt the self-incompatibility mechanism (Pearson, 1983). Additionally, alternative methods are employed, including end-of-season pollination, exposure to high humidity, surgical techniques, irradiation, double pollination, and pollination at elevated temperatures, among others.

Utilization of SI line

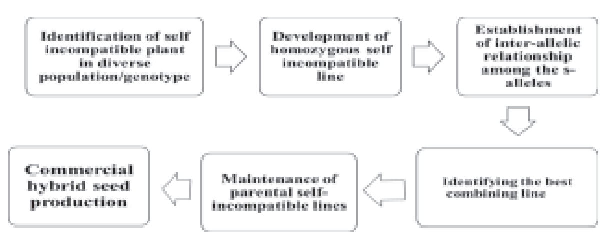

Fig.6: Basic steps in sporophytic self-incompatibility

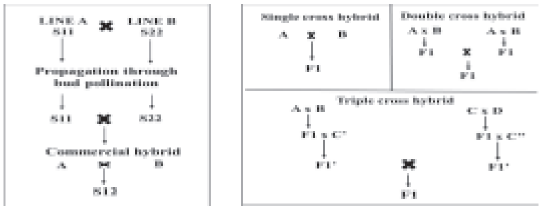

Fig. 7 : Use SI for hybrid production

Among cole vegetables such as cabbage, cauliflower, and broccoli, the sporophytic self-incompatibility mechanism is employed for hybrid seed production in various regions, including India (Singh, 2000). In hybrid seed production, it is essential for both parental inbreds to possess two different S alleles to ensure strong self-incompatibility, particularly in the case of single-cross hybrids. One of the self-incompatible inbreds is utilized as the female parent, while a suitable pollinator, often an open-pollinated variety, serves as the male parent to develop top-cross hybrids. Alternatively, for double-cross hybrids, four self-incompatible inbreds with distinct S alleles are employed.

1.3 Introduction: Genetic materials sourced from external populations undergo rigorous evaluation for desirable traits. Selected specimens are subjected to progeny testing and subsequent purification to ensure adaptability to local environmental conditions. Golden Acre cabbage, scientifically known as *Brassica oleracea* var. *capitata* 'Golden Acre', is a cultivar of cabbage native to the United States. White Vienna and Purple Vienna knol kohl, scientifically termed *Brassica oleracea* var. *gongylodes* 'White Vienna' and 'Purple Vienna' respectively, originate from Austria and belong to the turnip cabbage subgroup.

Table : 3 Cole crops hybrid developed in India by using cytoplasmic male sterility and self-incompatible

Crop mechanism (%)	Name of hybrid	Type to genetic	Developing Institute
Cabbage hybrid 1	KGMR-1(Pusa cabbage (KGMR-1=83-1-621 × GA-111)	Self -incompatibility	IARI, Regional station Katrain
Cabbage	Pusa cabbage hybrid 81 Pusa cabbage hybrid 822	CMS	IARI, New Delhi
Red Cabbage cabbage hybrid 1	Pusa red	CMS	IARI, New Delhi
Cauliflower	Pusa snowball hybrid 1, Pusa snowball hybrid 2, Pusa hybrid 301	CMS	IARI, New Delhi
Cauliflower	Pusa Kartik Shankar, Pusa hybrid 2	Self-incompatibility	IARI, New Delhi

1.4 Mass Selection: This method entails the identification and selection of superior individual plants solely based on observable phenotypic traits. The seeds from these elite individuals are pooled to create the next generation, with the success of selection contingent upon the heritability of the chosen traits. Pride of India cabbage variety from IARI Katrain is a selection of Copenhagen Market. Pusa Sharad, Pusa Meghna, and Pusa Deepali cauliflower are IARI-developed varieties. Pusa Broccoli KTS 1 from IARI Katrain is bred from exotic material.

1.5 Family Breeding: Progenies are meticulously evaluated over several successive generations, with multiple rounds of selection aimed at refining desirable characteristics. This method, widely utilized in beet breeding, holds promise for adaptation in other cruciferous crops like radish, carrot, and cauliflower.

1.6 Pedigree Method: The pedigree method initiates with the crossbreeding of two genotypes possessing complementary desirable traits. If the original parents lack all desired characteristics, a third parent can be introduced by crossing it with one of the hybrid progenies from the first generation (F_1). Superior types are meticulously selected across successive generations, while maintaining detailed records of parent-progeny relationships. Visual selection reduces the number of families to manageable levels, typically by the F_7 or F_8 generation, before precise evaluation for performance and quality ensues. Final evaluation involves multi-year, multi-location observations to identify potential weaknesses, rigorous yield testing, and quality assessment. Selection 8 (Pusa Mukta), bred from EC 10109 X EC 24855 to resist Black rot, exemplifies this approach, developed at Katrain.

1.7 Recurrent Selection: Heterozygous source populations serve as the foundation for recurrent selection techniques, which involve iterative cycles of selection to enhance genetic combining ability. Various iterations of recurrent selection may focus on general or specific combining ability, or incorporate reciprocal breeding strategies.

1.8 Heterosis Breeding: Hybridization strategies exploit the phenomenon of dominance variance to produce offspring with superior traits. Recent advancements in breeding methodologies have seen increased emphasis on developing hybrid varieties and disease-resistant cultivars in response to the burgeoning export potential of vegetables, particularly in regions like India. Pusa Kartik Sankar cauliflower, cultivated at IARI in New Delhi, is scientifically known as *Brassica oleracea* var. *botrytis* 'Pusa Kartik Sankar'.

2. Non-conventional Breeding Methods

Non-conventional approaches leverage advancements in cell and tissue culture techniques to manipulate plant genetic material outside the realm of sexual reproduction. These methods offer precise control over genetic traits without the reliance on traditional breeding cycles.

2.1 Mutation Breeding

Mutational breeding emerges as a potent tool for enhancing plant varieties with desired traits, benefitting both food crops and horticulture alike. Induced mutations serve a crucial role in conserving and preserving crop biodiversity. This method entails selecting mutants exhibiting desired traits in the M1 or M2 generation post-treatment with mutagens, subsequently releasing them as new cultivars after thorough evaluation and trials. For instance, mutant 19P-2 derived from variety Kjure17, induced by irradiating seeds with 60 kr gamma rays, exhibited semi-sterility. Mutation breeding serves to rectify defects and induce traits such as male sterility.

2.2 Tissue culture approaches

2.2.1 Plant tissue culture in crop improvement

Tissue culture has revolutionized plant genetic modification, with regeneration protocols being pivotal for successful genetic transformation. Studies by Abeyawardana and Koudela (2019) and Munshi *et al.* (2007) demonstrated successful regeneration of cabbage using shoot tips, hypocotyls, and cotyledons as explants, highlighting the importance of tissue culture in mass production of virus-free and disease-resistant cabbage plants. Gambhir *et al.* (2017) and Pavlovic *et al.* (2010) optimized regeneration protocols, emphasizing the significance of explant selection and hormone supplementation. Moreover, Lv*et al.* (2014) explored sexual hybridization and embryo rescue methods for intergeneric hybridization, while Li *et al.* (2017) and Pilih*et al.* (2018) focused on doubled haploid production for trait fixation and varietal improvement in cabbage. Mutagenesis studies by Ferrie *et al*. (2008), McClinchey and Kott (2008), and Liu *et al.* (2005) underscored the potential of in vitro techniques in generating novel genetic variation. These advancements highlight the critical role of tissue culture in enhancing genetic diversity and accelerating cabbage breeding programs.

2.2.2 *In vitro* grafting

In vitro grafting has emerged as a promising technique for generating chimeras in cabbage, enhancing genetic diversity and exploring interspecific hybridization. Studies by Noguchi *et al.* (1992), Hirata *et al.* (2000), and Chen *et al.* (2006) demonstrated successful chimeral shoot formation between cabbage and related species using tissue culture-based grafting methods. Optimal hormone concentrations and grafting techniques were employed to induce chimeral bud formation and root development. Despite its potential, in vitro grafting has not yet been widely adopted for commercial cabbage improvement. Further research is warranted to explore its utility in enhancing cabbage yield and stress tolerance through chimeral manipulation.

2.2.3 Somatic hybridization

Somatic hybridization, an in vitro fusion of protoplasts, offers a valuable avenue for generating soma-clonal variation in breeding programs. It enables the creation of intergeneric and interspecific hybrids, overcoming traditional breeding limitations. Studies by Sedlak *et al.* (2022) and others have demonstrated its efficacy in transferring desirable traits such as disease resistance and tolerance to environmental stresses between Brassica species. For instance, Ren *et al.* (2000) successfully introgressed bacterial soft rot resistance from *B. rapa* into *B. oleracea*, while Hansen (1998) incorporated resistance to black leg disease from *Camelina sativa* into *B. oleracea* via protoplast fusion, confirmed through RAPD marker analysis. Hu *et al.* (2002) utilized somatic hybridization to transfer black leg disease resistance from *B. napus* to hybrids involving Sinapis arvensis.

2.3 Biotechnology approaches in cole crops

2.3.1 Molecular-assisted breeding

Molecular-assisted breeding has transformed crop improvement by utilizing markers such as SSRs, SNPs, and InDels, enabling precise trait selection and gene introgression. In cabbage, DNA markers are instrumental in assessing genetic diversity and identifying traits like glucosinolate content. For instance, Wang *et al*. (2012)

developed a linkage map using SSRs and SNPs, crucial for molecular breeding and gene mapping. Genetic markers also facilitate the mapping of complex traits such as glucosinolate synthesis genes (Li and Quiros, 2002). Marker-assisted selection has been pivotal in developing disease-resistant cultivars, like clubroot-resistant Brassica lines (Li *et al.,* 2022). Molecular markers associated with traits like Xcc resistance and downy mildew resistance have been identified, enhancing breeding efficiency (Kifuji *et al.,* 2013; Giovanelli *et al.*, 2002). Moreover, marker systems such as RAPD, ISSR, SRAP, and SSR ensure the genetic purity of F1 hybrid seeds, aiding in seed quality assessment (Liu *et al.,* 2007). Comparative genomic analyses between Brassica and Arabidopsis provide valuable insights into gene evolution and aid in gene isolation efforts (Schmidt, 2002; Zhang *et al.,* 2019). Next-generation sequencing technologies have further accelerated genomic research in cabbage, promising continued advancements in molecular breeding strategies.

2.3.2 Crop improvement by genetic engineering

The introduction of transgenic crops, such as Bt cabbage, has significantly increased agricultural productivity and profits (Kumar *et al.,* 2020). By incorporating the cry1A (b) gene from *Bacillus thuringiensis* into cabbage, farmers have experienced reduced damage from lepidopteran insects like the diamondback moth (Kain *et al.,* 2022). This genetic modification effectively controls pests, decreases the need for chemical insecticides, and enhances crop yield and quality (Sehrawat *et al.,* 2021). Various studies have confirmed the efficacy of transgenic cabbage in resisting pests and diseases, including black rot.

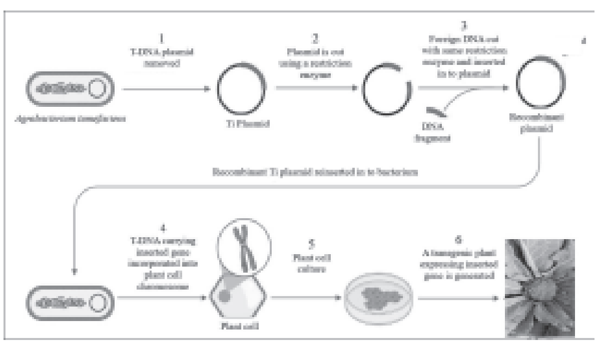

Fig.8: Agrobacterium mediated gene transfer

Moreover, genetic engineering has enabled the development of transgenic cabbages resistant to abiotic stresses like heat and salinity (Bisbis *et al.,* 2019; Zhang *et al.,* 2019). Through techniques like Agrobacterium-mediated transformation, genes encoding stress-responsive proteins have been introduced into cabbage cultivars, enhancing their tolerance to environmental challenges (Yarra and Xue, 2020). These genetically modified cabbages exhibit improved growth performance and increased stress tolerance compared to their non-transgenic counterparts. Additionally, research on the toxicity of recombinant scorpion insect toxin (AaIT) has shown promising results for enhancing insect resistance in cabbage (GreenMedInfo, 2013). AaIT demonstrates selective toxicity against insect cells while being harmless to human cells, suggesting its potential for developing transgenic cabbages with enhanced insect resistance. Overall, genetic engineering offers innovative solutions for addressing agricultural challenges, leading to the development of transgenic cabbages with improved traits such as pest resistance and stress tolerance, ultimately contributing to sustainable agriculture practices (Ahmar *et al.,*2019).

2.3.3 Genome and gene editing in cole crops

Traditional breeding methods like backcrossing and selection are time-consuming and resource-intensive. However, RNA interference (RNAi) and CRISPR-Cas9 technology offer rapid and precise ways to improve crop traits such as quality, yield, nutritional content, and stress tolerance (Rosa *et al.,* 2018). RNAi functions by silencing specific target genes through the production of small RNA molecules, enabling efficient genetic improvement in plants with low transformation rates. In cabbage, RNAi has been successfully utilized to delay bolting and enhance drought resistance (Xia *et al.* 2007).

CRISPR-Cas9, a revolutionary genome-editing tool, allows for the precise introduction of mutations into the genome of plants (Jinek *et al.,* 2012). This technology has shown promise in enhancing various traits in crops,

Table : 4 Study showing genome editing in cole crops (Jabeen *et al.,* 2024)

Target gene	Transformation method	Trait modification	References
BrDST71	Agrobacterium mediated transformation	Drought tolerance	Park *et al.,* 2018
CO 17–24	Agrobacterium mediated transformation	Resistance to mite	Shin *et al.,* 2020
BrVRN1	Agrobacterium mediated transformation	Delayed flowering	Hong *et al.,* 2021
BRGI	Agrobacterium mediated transformation	Regulation of glucosinolates	Kim *et al.,* 2021

including nutritional content and resistance to stresses (Jaganathan *et al.,* 2018; Das *et al.,* 2019). In cabbage, CRISPR-Cas9 has been employed to knockout specific genes associated with flowering time regulation, resulting in delayed flowering traits that contribute to improved crop quality and yield. Additionally, CRISPR-Cas9 has facilitated the development of early-flowering Chinese cabbage varieties independent of vernalization, addressing challenges associated with temperature sensitivity during seed germination (Jeong *et al.,* 2019). Despite challenges, such as lower research focus compared to other cruciferous crops, efficient genome editing in cabbage has been achieved using CRISPR-Cas9, demonstrating its potential for accelerating the development of improved cabbage varieties.

Conclusion

The cole crops benefit from a combination of conventional and modern breeding techniques, each offering unique advantages in enhancing traits vital for agricultural productivity and nutritional quality. While conventional methods have historically provided a foundation for crop improvement, modern techniques such as molecular breeding and genetic engineering accelerate the breeding process, enabling precise trait selection and novel trait incorporation. The synergy between these approaches holds promise for addressing evolving agricultural challenges and meeting the demands of a growing population. Embracing both conventional and modern breeding strategies is essential for ensuring sustainable cole crop production and resilience in the face of future agricultural needs.

Future thrust

Future thrust in conventional and modern breeding techniques for cole crops will focus on enhancing efficiency, precision, and sustainability. Conventional breeding will integrate advanced phenotyping, genomic selection, and accelerated breeding strategies to expedite trait introgression. Modern techniques will leverage high-throughput sequencing, CRISPR-Cas9 gene editing, and genomic prediction for precise trait manipulation and novel trait development. Furthermore, there will be a concerted effort to harness natural genetic diversity, explore wild germplasm, and address emerging challenges such as climate resilience and nutritional quality. Collaborative research initiatives and robust regulatory frameworks will facilitate the responsible deployment of these innovative breeding approaches to meet evolving consumer demands and global food security needs.

Data availability

No data available

Declaration statement

Reference

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