

## Advances in Breeding of Cole Crops: Cabbage

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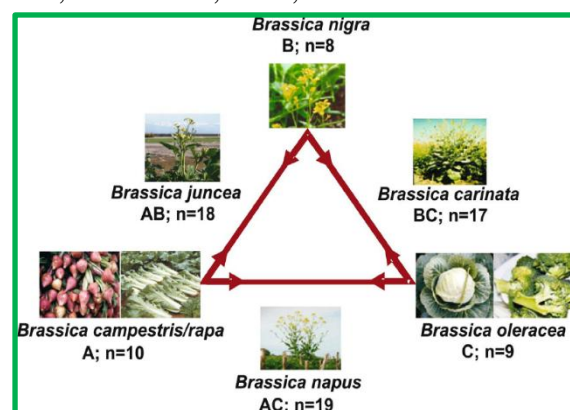
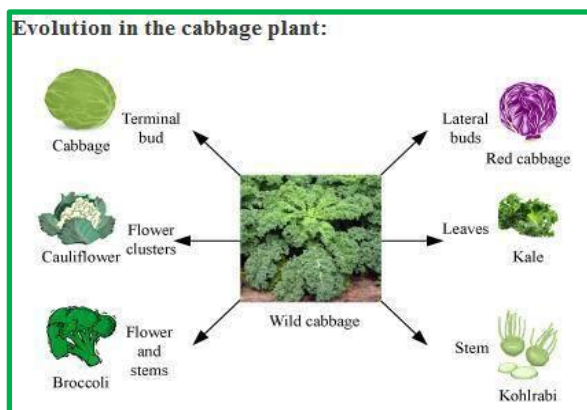
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**"When growing a member of the Cruciferae family the saying "what you see is what you get (to eat)" truly applies!"**

The terms "cold" and "cole" sound the same but have different meanings. "Cold" refers to temperature. "Cole" refers to any of various plants belonging to the Cruciferae or mustard family. The mustard family includes cool season crops such as Brussels sprout, cabbage, cauliflower. The close kinship of these crops enable diversified usage of plant parts. The flowers, seeds, stalks, and tender leaves of many species of *Brassica* can be eaten raw or cooked. Almost all parts of some species have been developed for food, including the root (rutabaga, turnip), stems - kohlrabi, leaves - cabbage, collard greens, kale, flowers - cauliflower, broccoli, buds - Brussels sprouts, cabbage, and seeds (many, including mustard seed, and oil-producing rapeseed). Cole crops are rich source of vitamins A and C. They also contain appreciable amount of minerals like phosphorus, potash, calcium, sodium and iron.

### Importance

- Culinary use- ranging from eating raw and simple steaming to pickling, stewing, braising.
- Low in carbohydrates, fats, calories. Good source of protein (balanced), minerals, vitamin A, vitamin C and vitamin D.
- Known for anticancer properties (-indole -3-carbinol).
- Cabbage juice: against poisonous mushroom.
- Consists of Flavonous compound : Sinigrin
- Includes antioxidants are ascorbic acid, tocopherols, carotenoids, isothiocyanates, indoles, flavanoids.
- As a medicinal herb to prevent the effects of alcohol, as compresses for ulcers and breast abscesses, treatments for rheumatism, sore throat, hoarseness, colic, and etc.



U's triagle by Nagaharu

**Cole Crops include:**Cabbage - *Brassica olerace var capitata*Kohl rabi - *Brassica oleracea var gongylodes*Cauliflower - *Brassica oleracea var botrytis*Broccoli - *Brassica oleracea var italica*Brussels Sprouts - *Brassica oleracea var gemmifera*Kale - *Brassica oleracea var acephala***Cole crops**

Crops	Scientific name	Chromosome no.(2n)	Type of pollination	Plant part used
Cabbage	<i>Brassica oleraceae (L.) var. capitata</i>	18	Cross pollination (73 %)	Head
Cauliflower	<i>Brassica oleraceae (L.) var. botrytis</i>	18	Cross pollination (70 %)	Curd
Knol khol	<i>Brassica oleraceae (L.) var. gongylodes</i>	18	Cross pollination (91 %)	Swollen stem
Brussels	<i>Brassica oleraceae (L.) var. gemmifera</i>	18	Cross pollination (72 %)	Sprouts
Sprouting broccoli	<i>Brassica oleraceae (L.) var. italica</i>	18	Cross pollination (95 %)	Flower heads
Kale	<i>Brassica oleraceae (L.) var. acephala</i>	18	Cross pollination (83 %)	Top leaves

**1. Cabbage**

The word cabbage was derived from the French word 'caboche', meaning head. The Latin name *Brassica* was derived from the Celtic word 'bresic' also meaning cabbage.

**Scientific classification****Kingdom** - Plantae**Phylum** - Tracheophytes**Class** - Angiosperms**Order** - Brassicales**Family** - Brassicaceae**Genus** - *Brassica***Species** - *oleracea***Binomial nomenclature** - *Brassica oleracea* L.**Chromosome number** -  $2n = 2x = 18$ **Origin** - Coastal area of the Mediterranean Sea.**Genome** - CC**Nutrient content**

- Cabbage: Excellent source of Vitamin C. In addition to containing some B vitamins

- Cabbage supplies some minerals like potassium, Phosphorus, sodium, calcium and iron into the diet.
- It provides the roughage that prevents constipation.
- Cabbage is consumed after cooking and fresh in salads.

#### Distribution

- Cabbage has worldwide distribution and is grown in Europe, India, Indonesia, Malaysia, Philippines, Central, East and West Africa, Central and South America and the Caribbean.
- Important cabbage growing countries include China, India, Russia, Republic of Korea, Japan, Poland, USA, Indonesia and Ukraine.
- **India is the second largest producer of cabbage in the world** with annual production of 60 lakh MT.
- Cabbage is the fourth most widely grown vegetable crop of India. Odisha, Bihar and Uttar Pradesh rank first, second and third in total area occupied, whereas, in total production Uttar Pradesh, Odisha and Bihar rank first, second and third respectively.
- The other important cabbage growing states are West Bengal, Assam, Maharashtra, Karnataka, Gujarat, Rajasthan, Uttar Pradesh and Haryana.

#### Botany of Cabbage

- The **inflorescence** may attend the length of **1-2 m**.
- The slender pedicels are **1.5- 2cm** long.
- Androecium: 6 stamens, Tetradynamous(4 long and 2 short)
- Gynoecium: 2 connate carpels, 2 stigma and false septum
- placentation: Parietal
- Fruit: Siliqua

#### Floral biology

- The buds open under pressure of rapidly growing petals and become fully expanded in about 12 hrs.
- Flowers are slightly protogynous (5 days earlier) and cabbage is naturally cross-pollinated due to sporophytic self-incompatibility.
- Bud pollination is effective to achieve selfing.
- For cross-pollination flower buds expected to open within 1-2 days are emasculated and are pollinated immediately with desired pollen using a brush/flower stamens.
- Cytoplasmic and genetic male sterility is also noticed in certain lines.
- Anthesis, or expansion of the floral parts, usually begins about 4 to 5 pm.
- The petals and stamens start to elongate and force the sepals apart.
- Early the next morning the petals have practically completed their growth and appear as a tightly rolled cylinder.
- Anthesis is completed by the reflexing of these petals to form the typical yellow cross-shaped flower.
- The pollen is released early in the first day the flower is open by a longitudinal split in the anther and a reflexing movement of the anther walls.
- This movement is not completed for several hours

#### Pollination and Pollen biology

- Insects visit the flower freely. Honeybees, although usually plentiful, often fail to be very efficient, because if they do not work at temperatures below 60° F.
- If Bumblebees are very plentiful in but usually a few of them are collecting pollen in nearby every field.

- Representatives of several families of solitary bees visit the flowers in search of both pollen and nectar. As these bees work at lower temperatures than honeybees, they are probably a very important factor in cabbage seed production.

### Self-incompatibility

- Homomorphic system type Sporophytic self incompatibility (SSI) *i.e.* in cabbage it is confirmed by (Adamson 1965). It is controlled by single s-locus with multiple allele but also reported more than one loci, more than 80 s-alleles reported in *Brassica* family.
- **A classical genetic analysis has grouped the *Brassica* S-alleles into two categories based on their phenotypic effect on self-incompatibility characteristics.**

### The compatible and incompatible reaction of pollen and stigma depends on

Genotypes (homozygous/heterozygous) of male and female plant at S locus. Interactions between the two *S* alleles

- Dominance ( $S1 > S2$ )
- Co-dominance ( $S1 = S2$ )
- Mutual weakening (no action by either allele)
- Intermediate gradation (1-100% activity by each allele)

### SI recognition is controlled by a multiallelic gene complex at a single locus, termed the *S*-locus

The S-locus consists of three genes

- ❑ 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)

Upon pollination, SP11 penetrates the papilla cell wall and binds SRK in an S-haplotype-specific manner This binding induces the autophosphorylation of SRK, triggering a signaling cascade that results in the rejection of self-pollen. MLPK (M-locus protein kinase) localizes papilla cell membrane and may form a signaling complex with SRK ARC1(Armadillo repeat-containing 1) identified through protein interaction with SRK. The proteasomal degradation of these substrates could result in pollen rejection.

### Public hybrids developed in Cabbage

Self-incompatibility		
Name of the hybrid	Type of Genetic Mechanism (Parentage)	Developing institution
KGMR-1, BRH-5	Self-Incompatibility (KGMR-1=83-1-621 x GA-111)	IARI regional station, Katrain
H-43,H-44	Self-Incompatibility (H-43=S2S2 x Pusa Mukta), (H-44=S2S2 x Cornell 83-6	IARI regional station, Katrain
	Male sterility	
KCH-5	Ogura CMS	IARI regional station, Katrain
H-11, H-46	CMS (H-11=Cornell 83-23 x golden acre), (H-46=Cornell 83-23 x golden acre)	IARI regional station, Katrain

### Male sterility

Genic male sterility has been reported in cabbage (Rundfeldt, 1960).

**Ogura CMS system (Intergeneric crosses):** Ogura male sterility was reported in Japanese radish, (Ogura 1968)

**CMS induced by interspecific cross:** *B. oleracea* with *rutabaga* – *B. napobrassica* (Chiang, Crete 1985, 1987) transfer resistance against *Plasmodiophora brassicae*.

EGMS is more popularly termed as “Two line Hybrid Breeding” as against “Three Line Hybrid Breeding” in case of CMS system. In Cabbage the TGMS mutant is activated at temperature  $<10^{\circ}\text{C}$ , Which makes the parent male sterile. (Rundfeldt, 1961).

### Classification

#### Based on variability

##### Based on Colour and form of heads

1. White cabbage : *B. oleracean* var. *Capitata* L. f. *alba*
2. Red cabbage : *B. oleracean* var. *capitata* L. f. *rubra*
3. Savoy cabbage : *B. oleraceae* var. *capitata* L. f. *sabauda*

##### Based on Place of origin:

1. Mediterranean cabbage : *B.O. var. capitata* ssp. *mediterranean*
2. Oriental cabbage: *B.O. var. cap. ssp. orientalis*
3. European cabbage. *B.O. var. cap. ssp. europea*

##### Based on Head shape:

1. Round shape: Eg. Golden Acre, Pride of India, Copenhagen Market
2. Flat head / drum head: Eg. Pusa Drum Head
3. Conical head: Eg. Jersey Wakefield
4. Savoy type: Eg. Chieftain

##### Myers classification based on size and shape of head

1. Wakefield and Winning Kadet group – Small, conical, early. Eg. Jersey Wakefield.
2. Copenhagen Market – Round, early, large heads. Eg. Copenhagen Market
3. Flat Head / Drum Head – Eg. Pusa Drum Head
4. Savoy group – Wrinkled leaves, high quality : Eg. Drum Head Savoy
5. Danish Ball head \_ Thin leaves, Compact head, fine texture. Eg. Danish Ball Head
6. Alpha group – Earliest group, very small head, solid. Eg. Miniature marrow
7. Volga group – Thick leaves, loose bottom Eg. Volga
8. Red cabbage : Similar to Danish ball head but have red colour leaves Eg. Red Rock.

### Breeding stations

Plant genetic resources are the backbone of any crop breeding programme. In 1982, the IPGRI has sponsored several missions to collect wild Brassica species.

1. Poly-tech University, Madrid, Spain
2. University of Tohoku, Sendai, Japan
3. Gene Bank of country from where samples are collected.

#### Research and breeding stations in India

1. IARI, Research Station., Katrain (Kullu Valley) H.P.
2. Dr. Y.S Parmar University of Horticulture and Forest, Solan. Kalpa.

### Breeding in India

The main purpose of breeding is due to self-incompatibility and poor yielding.

Reason for low yield:

1. Poor quality seeds
2. Lack of suitable cultivars for mild winter.
3. Lack of suitable agro techniques.

The self-incompatibility is used to produce hybrid seeds in cabbage and other cole crops, namely, cauliflower, broccoli, Brussels sprouts, and kale. The individual plants are self-pollinated through bud-pollination. Selection is applied for desirable characters and

strong level of self-incompatibility. This way several self-incompatible, but cross-compatible inbreds having different S-alleles are developed. Cabbage was introduced much earlier than cauliflower by **Portuguese**. It was grown during **Mughal period**.

From below, the resultant inbred lines such as  $S_1S_1$  and  $S_2S_2$  lines are planted in alternate rows in isolation and seed set on each line will be mostly hybrid seed where cross-fertilization is brought about by pollinating insects, mostly bees. The cross compatibility between inbreds of  $S_1S_1$  and  $S_2S_2$  assures the production of  $F_1$  hybrid seed.

### The kinds of Cabbage hybrids are

1. **Single Cross** - ( $S_1S_1 \times S_2S_2$ )
2. **Double Cross** - ( $S_1S_1 \times S_2S_2$ ) X ( $S_3S_3 \times S_4S_4$ )
3. **Top Cross** -  $S_1S_1 / S_2S_2 / S_3S_3 / S_4S_4$  X OPV

### Breeding objectives

1. High yield
2. Longer staying capacity in field after head formation/greater field holding capacity
3. Desirable head weight (1 – 1.5 kg)
4. Early head formation/early maturity
5. Storage ability (Storability)
6. Head shape and colour as per preference of consumers (essentially round heads, light green-green colour)
7. Less proportion of outer/wrapper leaves
8. Short and narrow cone
9. Firm head with short internal stem
10. Ability to tolerate frost
11. Resistance to diseases:
  - i. Black rot (*Xanthomonas campestris*)
  - ii. Alternaria leaf spot (*Alternaria* ssp.)
  - iii. Black leg (*Leptosphaeria maculans*)
12. Tolerance to insect – Pests

Diamond back moth (*Plutella xylostella*), cabbage moth (*Mamestra brassicae*), cabbage root fly (*Delia radicum*), cabbage maggot (*Hylemya brassicae*) and cabbage white butterfly (*Pieris rapae*)

### Methods of breeding in cabbage

#### 1. Conventional Breeding methods

In conventional breeding, progeny inherit genes for both desirable and undesirable traits from both parents. Breeders conserve desired characteristics and suppress undesirable ones by repeatedly selecting meritorious individuals from each generation to be the parents of the next.

#### 2. Non-conventional Breeding methods

In non-conventional breeding, encompasses essential all cell and tissue culture technique that assist in propagating studying and manipulating the plant gene without use of sexual cycle.

Cabbage is a highly cross pollinated crops. Following breeding methods are used for cabbage.

**A. Conventional Methods**

1. Introduction
2. Mass selection
3. Family breeding
4. Recurrent selection
5. Heterosis Breeding
6. Pedigree method
7. Bulk method

**B. Biotechnological Approaches**

1. Plant tissue culture
2. Genetic Engineering

Gene transfer through hybridization

- a) Interspecific and intraspecific gene transfer
- b) Gene transfer by non-sexual methods
- c) Gene transfer by manipulating DNA directly
- d) Agrobacterium mediated gene transfer

**C. Mutagenesis and crop improvement****D. RNA interference****E. RNAi for male sterility****F. Next generation sequencing****G. Bioinformatics tools in crop improvement****H. Nanotechnology in crop improvement****Introduction**

Introduced materials may be valuable in selecting desirable plants. In case of non-uniform introductions the desirable plants can be selected, their progeny increased, purified and later tested against the standard or local varieties for selecting the most promising lines. The chances of an introduction depends to a great extent upon the relationship between the agro climates, particularly temperature and day length of the donor and receptor areas. Promising in India – Golden Acre, August, Copenhagen Market.

**Mass selection:**

In this method the best individual plants are selected in the population and their seeds are composited for following generation. Since the mass selection is made exclusively based on the phenotype of the plants without any progeny testing, the success of selection depends upon the heritability of the characters under selection.

Characters which can be improved are

1. Maturity: days to 50 per cent heading (earliness)
2. Stalk length
3. Number of non wrapper leaves
4. Frame or plant spread
5. Shape of head – polar and equatorial diameter. Normal or spherical head: 0.8- 1.0 shape index(SI). Drum head ; SI < 0.5, conical head SI > 1.0
6. Compactness of head – associated with short sized core
7. Net weight of head < 750 g
8. Number of marketable heads
9. Yield

**Family breeding**

There is elaborate testing of progenies not only in F1 generation but also in later 2 or 3 generations and usually more than one cycle of selection. This method is practised in beet and can also be adopted in radish, carrot and cauliflower.

**Recurrent selection**

The source population of recurrent selection is heterozygous. It may be an open pollinated variety, single cross or double cross F1 hybrid, intercrossed progenies of selected inbred lines, a synthetic variety or a composite. There are four types of recurrent selection

viz. simple recurrent selection, recurrent selection for general combining ability and recurrent selection for specific combining ability, recurrent selection for general and specific combining ability and reciprocal recurrent selection.

### **Heterosis breeding**

The hybrid varieties are developed by exploiting the dominance variance in heterosis breeding. Hybrids are available for commercial cultivation in all the cross pollinated crops like cole crops and cucurbits etc. For the last two decades, vegetable breeders in India diverted their attention toward the development of hybrids and disease resistant varieties/hybrids. Vegetables have great potential for export and after value addition these can become an important commodity of agricultural export out of India.

### **Pedigree method**

It starts with the crossing of two genotypes, each of which have one or more desirable characters lacked by the other. If the two original parents do not provide all of the desired characters, a third parent can be included by crossing it to one of the hybrid progeny of the first generation (F1). In the pedigree method superior types are selected in successive generations, and a record is maintained of parent–progeny relationships. When the number of families has been reduced to manageable proportions by visual selection, usually by the F7 or F8 generation, precise evaluation for performance and quality begins. The final evaluation of promising strains involves (1) observation, usually in a number of years and locations, to detect weaknesses that may not have appeared previously; (2) precise yield testing; and (3) quality testing. Selection 8 (Pusa Mukta) is a selection from EC 10109 X EC 24855, resistant to Black rot. Developed at Katrain.

### **The bulk-population method**

The breeding differs from the pedigree method primarily in the handling of generations following hybridization. The F2 generation is sown at normal commercial planting rates in a large plot. Two types of artificial selection also are often applied: (1) destruction of plants that carry undesirable major genes and (2) mass techniques such as harvesting when only part of the seeds are mature to select for early maturing plants or the use of screens to select for increased seed size. The chief advantage of the bulk population method is that it allows the breeder to handle very large numbers of individuals inexpensively.

## **Biotechnological Approaches in Cabbage**

### **1. Plant tissue culture in crop improvement**

- The things required for the plant tissue culture are the plant tissues (explants), medium containing organic and inorganic compounds on which the plant could grow and develop.
- A high amount of growth hormones particularly Auxin and cytokinin.
- Crops used to produce from this technology facilitate the interspecific and intergeneric crosses to overcome physiological based self-incompatibility. A vast variety of crops has been recovered through IVF via pollination of pistils and self and cross pollination of ovules.
- the use of delayed pollination, distant hybridization, pollination with abortive and irradiated pollen and physical and chemical treatment of host ovary have been used to implied haploidy.
- Embryo culture is another kind used to make crops valuable.
- These technologies could easily simplify breeding programs and overcome some important economical and agronomic traits that would never be produced from conventional ways of plant breeding and plant improvement.



## 2. Crop improvement by genetic engineering

- Gene transfer between unrelated species of plants has been playing a very crucial role in Cole crop improvement.
- By transforming genes many useful traits like resistance to insects, stress and disease has been transferred to many crop varieties from non-cultivated plants.
- Recombinant DNA methods and many other methods are in use for transformation of genetic information.
- Genetic engineering is a DNA recombination technique that has made possible gene transfer between dissimilar genera or species.

Secondly, as it avoids the problem of linkage drag associated with the conventional breeding it is more effective and it is less time consuming. Till now, many genetic engineered crops have been developed and commercialized that result in improved production efficiency, increased market focus and enhanced environmental conservation. Such crops include longer post-harvest storage insect resistance cabbage.

### Gene transfer through hybridization

- I. **Plant breeding by intraspecific gene transfer**
- II. **Interspecific gene transfer**
- III. **Gene transfer by non-sexual methods** (cell, tissues, recombinant DNA and cell fusion methods)
- IV. **Gene transfer by manipulating DNA directly**
- V. ***Agrobacterium-mediated gene transfer***

### Mutagenesis and crop improvement

- Mutational breeding is powerful tool for raising plant varieties with desired traits with equally beneficial to food crop as well horticulture.
- Induced mutations can play an important role in the conservation and preservation of crop biodiversity.
- In this approach, mutants with desired traits were selected in the M1 or M2 generation after treatment with mutagens and then released as new variety for cultivation after evaluation and trials.
- **Mutant 19P-2 of variety Kjure17** was induced by irradiating seeds with **60 kr** gamma rays was semi sterile.
- Mutation breeding is resorted to **eliminate defects** and to induce characters like **male sterility**.

### RNA Interference

RNA interference is an emerging tool in biotechnology for crop improvement. It has been widely used for increasing crop yield, resistance against biotic and Abiotic stresses. RNAi has also been used for generating sterility in seeds and producing hybrid seed. Genes that involved in pollen production can be targeted by RNAi. RNAi has also been used for generating sterility in seeds and producing hybrid seed. Genes that involved in pollen production can be targeted by RNAi. A male sterile tobacco line has been developed by targeting the expression of TA29, a gene necessary for pollen development. Male sterility is also generated by RNAi by controlling the Msh1 gene expression in tobacco and tomato that result rearrangements in the mitochondrial DNA that is associated with natural cytoplasm male sterility.

### Next-generation sequencing

Next-generation sequencing (NGS) technology is the cutting-edge technology for genome sequencing of several species. It has been proved an essential gadget for development of novel or atypical molecular markers and determining genes of agricultural importance.

### Bioinformatics

Bioinformatics resources in addition to different web databases are providing vast information about the genomic data that is largely required for the research purpose. The bioinformatics is providing crucial information about the genomic data of crops and the sequence of many genes are being explored by this technology. This could possibly help us to sequence the crops which is economically important and the traits that are more beneficial.

### Nano-particles

The positive effects observed by using these Nano-particles include enhanced germination, enhanced length of roots and shoots, and increased vegetative biomass of seedlings in many crops. TiO<sub>2</sub> Nano-particles have been known to enhance the growth of Cabbage

### Seed production in Cabbage

#### a) Stump method

- Heads examined for trueness to type. Heads are cut just below the base by means of sharp knife, keeping the stem with outer whorl of leaves. Beheaded portion of plant is called stump.
- After dormancy is broken, buds sprout from axils of all leaves and leaf scars.

#### Advantages:

- Extra income
- Matures 12 - 15 days earlier.
- Seed yield is increased.

#### Disadvantages:

- Flowering shoots are decumbent and requires heavy stacking.

#### b) Stump with central core intact method

- Heads are chopped on all sides with downward perpendicular cuts in such a way that central core is not damaged.
- Improvement over stump method.
- During last week of February and upto 15th March when head starts bursting, 2 vertical cuts are given. (Central growing part not injured)
- In absence of such cuts, heads burst out irregularly and sometimes growing tip is broken.

#### Advantages:

- Very heavy stacking is not necessary.
- Seed yield is increased.

#### Disadvantages:

- Chopped heads cannot be marketed.

#### c) Head intact method

- Head is kept intact and only a cross-cut is given to facilitate the emergence of stalk.

#### Advantages:

- Removal of heads or chopping of heads on all sides is not required.
- Very heavy stacking is not required.

#### Disadvantages:

- Seed yield is slightly lower as compared to stump, or stump with central core intact method.

**Few breeding achievements**

Diseases	Resistant variety
Tip burn (non-parasitic )	Wisconsin ,Copenhagen, Bonaza
Downey mildew( <i>Peronospora parasitica</i> )	january king
Yellows ( <i>Fusarium oxysporum</i> )	Wisconsin ball head ,improved wisconsin, ball head, marion market
Club root ( <i>Plasmadiophora brassica</i> )	Miher, late moscow, white russian
Powdery mildew( <i>Mycosphaella</i> )	louisiana
Black rot ( <i>Xanthomonas campestris</i> )	Pusa mukta , louisiana
Mosaic ( several viruses)	Badger ball head

**Wild species commercially exploited for breeding**

Sources of traits	Species
High Glucoraphanin	<i>Brassica villosa</i>
Resistant to cabbage Aphid	<i>B. incana</i> , <i>B. villosa</i>
Resistance to cabbage white fly	<i>B. cretica</i> , <i>B. incana</i>
Resistance to black rot	<i>B. carinata</i>
Resistance to Sclerotinia	<i>B. incana</i>

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