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Research Progress on Polyploidy Mutagenesis of Chinese Cabbage Vegetables

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Abstract

Chinese cabbage is native to China, divided into Chinese cabbage and non-heading Chinese cabbage, Chinese cabbage is also known as celery cabbage, non-heading Chinese cabbage is also known as pakchoi, garden sass, flowering Chinese cabbage, etc.; it is one of the main leafy vegetables in various parts of China, and occupies an important position in vegetable production and market supply. Polyploid plants are morphologically enormous, manifested by the enlargement of roots, stems, leaves, and flowers. Chinese cabbage vegetables mainly use tender leaf clusters, leaf bulbs, flowers or young stems as edible organs, and the huge and tender edible organs are exactly what consumers need. The characteristics of the morphological giganticity of polyploid plants are particularly important in the performance of Chinese cabbage, and they are also of the most development value. In this paper, the overview of Chinese cabbage multiplication sports species, the method of colchicine induction and polyploidy of Chinese Cabbage species, the identification method of polyploidy and the characteristics of Chinese cabbage polyploidy are summarized, and the research and application potential of polyploidy induction technology in cabbage breeding is discussed. Compared with diploid plants, Chinese cabbage tetraploid plants are heavier, the leaves are enlarged, and the leaves are more rounded, so that the Chinese cabbage varieties are more robust, the yield is higher, and it can also meet the consumption needs of the people. The quality of tetraploidy in Chinese cabbage is better, and the content of chlorophyll, soluble protein, amino acids, vitamin C and soluble sugars is significantly increased compared with diploid, and the content of crude fiber is decreased. And tetraploid Chinese cabbage and diploid hybridization is not affinity, will not be naturally hybridized with other diploid Chinese cabbage, easy to keep seeds and pure. Therefore, it is of great significance to mutagenic Chinese cabbage polyploidy, cultivate new varieties of Chinese cabbage polyploidy, and promote and apply in production.

Keywords

Chinese cabbage; Polyploidy; Mutagenesis

Cabbage belongs to the Brassica genus of the Cruciferae family (*Brassica)* and *the* Brassica species (*B. campestris Chine n s is* L), annual or biennial herbaceous plants, including Chinese cabbage subspecies (cabbage subspecies) [*B. campestris L. ssp. pekinensis* (Lour) Olsson] and Brassica rapa subsp. (non-heading Brassica rapa subsp.) [*B. campestris ssp. Chinensis* Makino, which originated in China. The edible parts of this type of vegetable are the soft and tender leaves, leaf heads, flower stalks or stems. They have a good flavor and are rich in various vitamins, minerals, and a small amount of protein, sugars, cellulose, etc. [1]. According to the horticultural classification method of "Chinese Vegetable Cultivation" (Second Edition), cabbage vegetables can be divided into Chinese cabbage (cabbing

cabbage) and Chinese cabbage (non-cabbing cabbage); Chinese cabbage (cabbing cabbage) is further divided into loose-leaf cabbage varieties, semi-cabbing varieties, flower-heart varieties and heading varieties; Chinese cabbage (non-cabbing cabbage) is further divided into ordinary cabbage, collapsed cabbage, stalks, stalks, multi-headed cabbage, rapeseed and other varieties [2]. The chromosomes of the subspecies and varieties of cabbage are all 2n = 2x= 20, and they can hybridize with each other. It is an important native vegetable of China, with rich germplasm resources and diverse varieties. It is widely distributed in China, with Chinese cabbage being the main crop in the north and Chinese cabbage being the main crop in the south. It is a vegetable that residents like to eat, and its planting area and consumption rank first among all types of vegetables in China.

1. Overview of Polyploid Breeding in Chinese Cabbage

According to statistics, approximately 30% to 35% of angiosperms and 70% of grasses in nature are polyploid plants. In order to improve plant varieties and promote their evolution, breeders have been keen on related research on polyploid induction breeding since the 1930s [3]. Cabbage is an important vegetable crop in the genus Brassica in the family Cruciferae. Cabbage has a small genome and is one of the vegetable crops suitable for polyploid breeding and genetic research. Therefore, polyploidy of cabbage has always been highly valued. Since the 1980s, (Liu Huiji et al. 1988, 1990, 1995, 2002) have used somatic cell chromosome artificial doubling methods to breed tetraploid Chinese cabbage varieties such as 'Suzhou Qing', 'Ai Jiao Huang', 'Han You 1' and 'Shu You 1' [4-7]; (Wang Zixin et al. 1992) and (Wang Yuhai et al. 2002) used naturally occurring 2n gametes to obtain tetraploid Chinese cabbage varieties such as 'Cui Bao', 'Cui Lu' and 'Duo Kang 6' through hybrid breeding [8]. In addition, cabbage production has always been seriously threatened by viral diseases. Currently, there are no cabbage varieties that are immune or highly resistant to viral diseases. However, different varieties have different resistance to viral diseases. Generally, those with darker color are more resistant than those with lighter color. The results of inducing polyploidy in vegetables such as watermelon, melon, and cucumber show that polyploid plants have thicker mesophyll tissue, higher phylloxin content, darker leaf color, larger and thicker leaves, and stronger disease resistance. Therefore, by carrying out polyploidy breeding of cabbage, it is possible to obtain disease-resistant cabbage varieties, which also meets the expectations of vegetable farmers for high yield and high efficiency of cabbage.

2. Method of Colchicine Induction of Polyploid Cabbage Varieties

Since the 1930s, breeders have been keen on polyploid induction breeding. In particular, after Blakeslee and Avery discovered in 1937 that colchicine could induce chromosome doubling, a craze for colchicine-induced polyploid breeding was launched. As early as 1974, someone used 1% colchicine plus lanolin ointment to treat the cabbage variety Qingmaye. There are two methods for inducing polyploidy using colchicine: solution immersion method and drip method. Cabbage seeds are generally induced by solution immersion method [8].

2.1 Colchicine induction

As early as the 1980s, (Liu Huiji et al. 1990) began to try to use colchicine to induce polyploidy in cabbage varieties. They used a 0.4% aqueous solution of colchicine plus an equal amount of lanolin to treat the cotyledon stage seedlings of dwarf yellow cabbage. After 15 days, they repeated the treatment for the second time and finally obtained four homologous tetraploid dwarf yellow cabbages with a mutation rate of 0.8% [9] (Zhang Hecheng et al. 1999). The method adopted was colchicine shoot tip treatment technology. Cabbage seedlings were treated with 0.1%-0.3% colchicine solution at 10℃ for 24h, 48h and 72h. After screening for appropriate concentrations and identification of the chromosome number of mutants, the final doubling success rate reached 35.74% [10]; (Hu Qiuwen et al. 2015) The excellent diploid late- bolting non-heading cabbage 'Wuyueman' was used as the experimental material. Colchicine at mass concentrations of 1.5 and 2.0 g/L was used to induce its shoot tip growth point. The treatment was carried out once in the morning and evening every day, and each time the drip treatment was performed 4 to 6 times, for a total of 4 treatments, with distilled water as the control. Among the four treatments, the best induction effect was achieved after 6 treatments with 2.0 g/L colchicine, with the highest tetraploid mutation rate reaching 5.90% [11]; (Pan Jingwen et al. 2020) In order to cultivate high-yield and high-quality non-heading cabbage tetraploid Suzhou Green, colchicine aqueous solutions with concentrations of 0.1%, 0.2% and 0.3% were used to treat the cotyledon growth points of Suzhou Green diploid seedlings, with distilled water dripping as the control. The treatment was carried out twice a day, 20 μL each time, for a total of 5 days. The experimental results showed that 0.2% had the

best doubling effect, and the tetraploid mutation rate reached 8.42% [12]. In the early days, colchicine was widely used for in vivo induction. This method is simple to operate and easy to handle, but it is difficult to obtain pure tetraploids through in vivo induction, and the incidence of chimeras is high.

2.2 Induction by colchicine seed treatment

There are several methods for treating materials with colchicine: dipping, dripping, smearing and injection. Among them, the dipping method is simple, easy to operate and has a high induction efficiency [13]. The method of directly soaking seeds in colchicine solutions of different concentrations has successfully induced polyploidy in many plants such as chrysanthemum and Chinese cabbage [14, 15]. (Liu Huiji et al. 1995) conducted experiments using two methods to induce tetraploidy in cabbage. The first method was to soak dry seeds with 0.2%-0.4% colchicine solution, treat them for 24-48 hours, then rinse them with clean water and sow them; the second method was to treat the seedling growth point with colchicine solution of the same concentration, once in the morning and afternoon every day for 3 consecutive days, and shade them during the treatment [16]. (Huang Yu et al. 2019) treated diploid germinating seeds of black cabbage with a concentration of 0.01% to 0.03% colchicine, and the treatment time was about 8 hours, which was more suitable. The mutation rate of tetraploid mutation was between 0.5% and 2.75% [17]. The disadvantage of using colchicine seed treatment induction method is that it is easy to cause seed deformity, reduce seed germination rate, and affect the differentiation rate of seedlings.

2.3 Colchicine tissue culture induction

(Zhang Jianjun et al. 2011) believed that treating the culture materials with colchicine in vitro culture could overcome the defects of traditional polyploid induction and greatly improve the success rate of polyploid induction. He used three methods of colchicine in vitro culture treatment: the first method was to treat the cotyledons with colchicine, soak the sterilized seeds in a 100-1000 mg/L colchicine solution for 24-48 h, and then take out the cotyledons for conventional tissue culture. The second method was to treat the callus with colchicine, take 2-3mm callus and transfer it to a liquid culture medium containing 100-500 mg/L colchicine, oscillate at 40r/min for 24-48 h, and then transfer it to a solid induction culture medium for conventional tissue culture. The third method was to treat the regenerated buds with colchicine, transfer the buds of about 5mm to a liquid bud induction culture medium containing 100-500 mg/L colchicine, and oscillate and culture them in the same way as the treatment of the callus. Experiments have shown that the best results are achieved when regenerated seedlings with 3-4 true leaves are treated with solid culture medium containing different concentrations of colchicine and then induced to produce tetraploid plants. The doubling induction rates of the two cabbages, 'Shicai' and 'Piaoercai', reached 13.33% and 6.52%, respectively [18]. The colchicine tissue culture method for inducing polyploid plants is easy to repeat, convenient to purify, has a low chimera incidence, and is fast in effectiveness.

2.4 Colchicine 2n gamete induction

The selection of 2n gametes and the inheritance of 2n gamete generation have been reported in potatoes (Xiao Zengkuan et al., 1986) and Chinese cabbage (Zhang Chenghe et al., 2007) [19]. The use of colchicine to induce 2n gametes to obtain polyploidy has been studied in Oriental lily (Zheng Sixiang et al., 2004) and poplar (Kang Xiangyang et al., 2004) [20, 21], but there are few reports on cabbage. The frequency of 2n gametes in cabbage is very low. (Zhong Cheng et al., 2010) The best effect of inducing 2n gametes was achieved by microinjecting 510μL of 0.20% colchicine into 3mm diploid cabbage buds once. The frequency of 2n gametes reached 20.20%, which is 3.63 times that of the control without colchicine treatment. Using a tetraploid male sterile line as the female parent and a diploid line with a high 2n gamete frequency and excellent economic traits as the male parent, sexual polyploidization hybridization was performed to obtain tetraploid new germplasm, providing a new method for polyploid breeding [22].

3. Characteristics of Polyploid Cabbage Varieties

3.1 Hugeness

Polyploid cabbage varieties show gigantism due to the doubling of chromosome sets. This gigantism is often manifested in the enlargement of roots, stems, flowers, and leaves, making the cabbage varieties more robust and producing higher yields. This can also meet the consumption habits of people in some areas who like to eat large vegetables.

Compared with diploid cabbage, the tetraploid black cabbage studied by (Liu Huiji et al. 1994) has thicker leaves, heavier single plants, and a 16.7% increase in yield [23]. The leaves of tetraploid cabbage studied by (Xie Weihua et al. 2002) also show this characteristic [24]. The gigantic morphology of polyploid plants is particularly important in cabbage and has the greatest development value. Cabbage is mainly eaten for its leaves, and huge, plump and tender leaves are exactly what consumers want.

3.2 Stress resistance

Polyploid cabbage varieties not only improve gene activity, but also enhance the ecological adaptability and stress resistance of the plants [25]. Polyploid cabbage varieties generally have strong plants and thick stems, and therefore have strong resistance to lodging. Some also have other resistances such as drought resistance, disease and insect pest resistance. (Liu Huiji et al. 1990, 1992, 1994) The heat-resistant and cold-resistant "Nannong Aijiaohuang", "Heyou 2" and "Hanyou 1" cabbage varieties have been successfully cultivated. (Zhang Zhenchao et al. 2007) Through experiments, it was found that the cold resistance of tetraploid non-heading cabbage is better than that of diploid cabbage. Tetraploid non-heading cabbage with excellent traits has been successfully cultivated [26]. These have strongly proved that the stress resistance of polyploid cabbage is stronger than that of diploid cabbage.

3.3 Low fertility

Sexuality refers to the ability of a plant to form normal reproductive organs or to flower and set seeds normally. The heterogeneity of autopolyploids is lower than that of diploids, which is mainly due to the disorder of chromosome pairing during meiosis in polyploids, which is mainly manifested in stunted seed development and decreased pollen viability. (Shang Aiqin et al. 1999) The seed setting rate of artificial selfing of induced tetraploid Chinese cabbage is significantly lower than that of diploid, reaching an average of 42.3%, while octoploid Chinese cabbage plants are almost sterile [27]. When odd-numbered polyploidy forms gametes through meiosis, the chromosomes cannot pair normally, resulting in plant sterility. Using this characteristic, seedless production can be carried out in the production of fruits and vegetables, such as triploidy. Seedless watermelon "Heimi No. 2", "Xuefeng Seedless", "Dongting Seedless" and other main watermelon varieties grown in China [28] can also be used in the production practice of triploid citrus, seedless grapes, apples, etc. use.

3.4 Increased nutritional content

The nutritional content of polyploid cabbage is significantly higher than that of diploid (Liu Huiji et al. 1990). The selected tetraploid non-heading cabbage "Nannong Dajiaohuang" significantly increased the content of VC, amino acids, Ca, P and Fe. content, the crude fiber content decreased by 12.7%, and the overall quality improved significantly [7] (Huang Yu et al. 2019). The contents of chlorophyll, soluble protein, VC and soluble sugar in the induced black cabbage tetraploid were significantly higher than those of the diploid, while the crude fiber content was significantly reduced, and the quality of the black cabbage tetraploid was significantly improved [17].

4. Methods for Identification of Polyploid Cabbage Varieties

In the process of polyploid induction in cabbage, timely and accurate identification of polyploid plants is an important step in polyploid breeding. Using appropriate identification methods can not only shorten the plant culture cycle, but also improve the effectiveness of polyploid breeding.

4.1 Morphological identification method

For polyploid cabbage materials induced by mutation, morphological observation is the most intuitive, simple and convenient identification method. The ploidy of the induced plants can be preliminarily identified by observing their external characteristics during the growth and development period. Polyploid plants generally have thick and short stems, slow growth and development, thicker leaves, darker leaf color, wider leaves, and a smaller leaf shape index. The appearance morphology of cabbage varieties is mainly identified in four stages: seedling stage, vegetative growth stage, flowering stage, and pod-setting stage. This method can reduce a lot of breeding workload, but its disadvantage is that there is a large empirical factor, which may vary from person to person, and the accuracy of identification is not high. (Du Xianming et al. 1995) By observing the morphology of tetraploid and diploid dwarf cabbage plants, it

was found that the cotyledons of tetraploid plants were significantly enlarged, and the appearance of the first true leaf was 1-2 days later than that of diploid plants. The flowering period of tetraploid dwarf yellow and polyploid dwarf white was 2-3 days later than that of their diploid counterparts [29].

4.2 Cytological identification

Ploidy of cabbage varieties can be screened and identified by measuring the average length of leaf stomata and the size of pollen grains and comparing the number of chloroplasts in stomatal guard cells. The method of identifying ploidy by using the characteristics of plant leaf stomata is simple and easy to operate. It only takes a few minutes to identify a material, which is a simple and efficient method for identifying ploidy. Compared with diploids, the average length of tetraploid stomata is longer, the pollen grains are generally larger, the number of chloroplasts in guard cells increases, and the traits are significantly changed (Xie Weihua, 2002). Induced cabbage found that the average length of tetraploid stomata was 34.6% longer than that of diploids, and the chlorophyll content of guard cells was 46.9% higher than that of diploids [24].

4.3 Flow cytometry identification

ploidy analyzer is used for identification, that is, the DNA content in the nucleus of a single cell in the plant leaf is quickly determined by flow cytometry, and the chromosome ploidy of the plant is identified based on the curve of the DNA content [30]. The flow cytometer has the characteristics of simple sample preparation, high sensitivity and resolution, good data repeatability, and fast testing speed. In addition, it can also quickly identify whether the cell is in the S phase, so the flow cytometer is particularly suitable for plant ploidy detection and analysis with a large number of samples. The disadvantage is that the flow cytometer is very expensive, the maintenance personnel need to have a high level of professionalism, and the instrument operation is complicated.

4.4 Direct chromosome counting method

Direct chromosome counting is the most intuitive method for identifying polyploidy. There are usually two methods for identifying plant chromosomes: the conventional chromosome pressing method and the wall-removing hypotonic method.

The pressing method generally uses the root tip, stem tip, callus and other parts of the plant with vigorous division as materials, and uses acetic acid magenta or carbofuxin to dye the pressed slices, so that more cells and chromosomes in the metaphase of mitosis can be observed. Good operating skills are required in the preparation of the slices. The wall removal and hypotonic method first removes the cell wall with cellulase and pectinase to obtain protoplasts, and then treats the cells with hypotonic solution. Treatment with hypotonic solution can increase the dispersion of chromosomes. Compared with the conventional pressing method, the wall removal and hypotonic method has many advantages. It can be used for chromosome counting, chromosome karyotype analysis, Gimsa banding and other studies. This method requires good experimental conditions and rich experience.

5. Application Potential of Polyploid Cabbage Varieties

Compared with diploid cabbage, tetraploid plants are heavier, have enlarged leaves, wider petioles, and more rounded leaves, all of which reflect the hugeness of tetraploid plants. The gigantic shape of polyploid plants is particularly important in cabbage. Cabbage mainly uses its leaves for food, and huge leaves are exactly what consumers need. The contents of chlorophyll, soluble protein, amino acids, VC and soluble sugar in tetraploid cabbage increased compared with diploid, while the crude fiber content decreased. Soluble protein, amino acids, soluble sugar, and vitamin C are all nutrients needed by the human body. The content of these in tetraploid cabbage is significantly increased, which is more conducive to the human body's intake of more nutrients and satisfies the quality of vegetables. Breeding expectations. Moreover, tetraploid cabbage is not compatible with diploid hybrids and will not naturally hybridize with other diploid cabbages, making it easy to retain seeds and maintain purity. It can be seen that breeding tetraploid cabbage can well solve the current problems in cabbage production.

With the continuous improvement of the living standards of urban and rural residents, the demand for vegetables is also increasing. Cabbage vegetables are the main varieties grown in autumn and winter, and their production requires large-scale planting planning. Therefore, it is imperative to cultivate different types of cabbage varieties that are suitable for different cultivation conditions and different destinations, such as high yield, high quality, disease and

pest resistance, cold resistance, and late bolting. The use of polyploid induction methods to improve the characteristics of cabbage varieties can accelerate the breeding process. At present, there have been many reports on the use of colchicine to induce tetraploid cabbage varieties, which provides a method for further theoretical research. A large number of cabbage tetraploid varieties that have been successfully induced have also been promoted and applied in production, and have achieved good results.

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