AGRICULTURAL SCIENCES

THE METHOD OF OBTAINING APOMICTIC SEEDS OF WHITE HEAD CABBAGE VARIETIES OF UKRAINIAN BREEDING

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At present, in varietal and hybrid breeding of white cabbage, a method of genetic stabilization of breeding material, which is quite laborious in terms of the number of operations and time duration, is used, which is based on forced self-pollination of varietal populations and their subsequent genetic analysis for homozygosity [1, c. 4]. Since white cabbage is a cross-pollinated crop, it is practically impossible to achieve absolute alignment of the lines in the above-mentioned way. Therefore, in order to increase the effectiveness of the selection process, there is an urgent need to develop more effective and accelerated methods of genetic stabilization of breeding-valuable material. From experimental practice, a biotechnological method of accelerated creation of homozygous forms of white cabbage obtained from haploid regenerants is known. This method involves the cultivation of isolated anthers on artificial nutrient media *in vitro* [2, c. 180]. The disadvantage of this method is that the formation of regenerants by the method of direct or indirect embryogenesis (through the passage of the callusogenesis stage) can occur from the somatic cells of the anther, which have a diploid set of chromosomes and do not ensure homozygosity of the obtained material.

In contrast to the above method, the use of induced apomixis practically excludes false results and guarantees 100% of obtaining diploid homozygotes. Irregular apomixis is a specific method of reproduction that allows obtaining absolutely identical copies of the mother form of plants. From a genetic point of view, this phenomenon consists in the development of a seed embryo from an egg cell or another cell of the embryo sac in the absence of pollination. This type of apomixis is called parthenogenesis or apogamy and occurs in nature with a rather low frequency of manifestation (10^{-6}) [3, c. 2]. Recently, attempts

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have been made by researchers to induce irregular apomixis in the most important agricultural plants. Unfortunately, most of them have almost no ability to apogamy.

On white cabbage, the method of induction of irregular apomixis was used by various researchers, while positive results were achieved in the case of using exogenous treatment of unfertilized ovaries with an aqueous mixture of growth regulators to stimulate mitotic division and doubling the number of chromosomes in unfertilized eggs [4, c. 711]. The same author showed that the initiation of parthenogenesis is largely influenced by the genotype of the plant, the stage of development of the embryo sac, the phytohormonal status of growth regulators (gibberellins or cytokinins), the weight ratio of the regulators in the apomictic agent, and the consumption rate of the agent per one unfertilized egg [4, c. 715].

In our research conducted during 2017–2021, various options for obtaining apomictic white cabbage seeds were tested. In particular, experiments were carried out that involved the treatment of castrated buds with an aqueous mixture of growth regulators (gibberellin and cytokinin). This method of pollination stimulated the exclusively partecarpic growth of pods without the formation of apomictic seed embryos in all studied varietal genotypes of white cabbage.

The purpose of the conducted research was to develop a more effective method of obtaining apomictic white cabbage seeds, in contrast to those already used in experimental practice.

In our research, conducted during 2017–2021 years different variants of getting apomictic seeds of cabbage were tested. For example, we conducted experiments, in which carried out the treatment of castrated buds with an aqueous mixture of gibberellins and cytokinin. This approach stimulated in all studied genotypes of white cabbage parthenocarpic growth only without forming apomictic seed embryos into pods. To achieve the goal, the following manipulations with plant material were carried out (Figure 1). In the morning hours (7–10 hours of the day) in the inflorescence of cabbage seeds, from which it is planned to obtain apomictic seeds, the opened flowers and buds were removed, leaving only those of them that should open in 2–3 days and in the ovaries of which, respectively, already fully formed embryo sacs are contained. Petals and anthers were removed with tweezers from the remaining buds, which at that time had not yet cracked and were not shedding their own mature pollen. The purity of the castration was monitored with a magnifying glass. To eliminate the possibility of fertile white cabbage pollen getting on the surface of the receptacles, the latter were treated with a 96% aqueous solution of ethyl alcohol. After treatment with ethyl alcohol, pollen from Chinese radish (loba) plants (*Raphanus sativus* L. species), which is incompatible with *Brassica*

oleracea var. *capitata* L. Then, with the help of a micropipette, 10–30 μl of an apomictic agent was applied to the base of the columns of castrated, unopened flowers, which consisted of an aqueous mixture of growth regulators – gibberellic action (gibberellic acid, commercial brand of the substance – GA_3) and cytokinin action (6-benzylaminopurine, commercial substance brand 6-BAP) in different weight ratios.

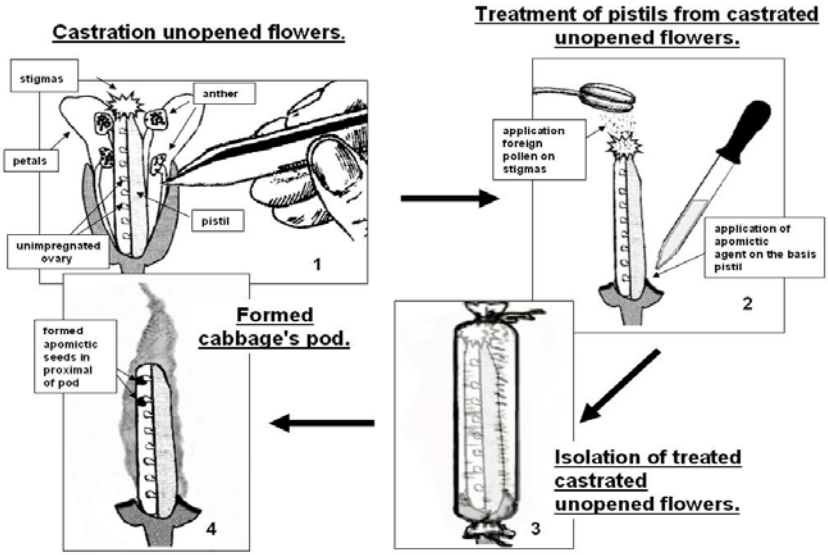

Figure 1. Manipulations with plant material

Castrated bud buds or branches with castrated bud buds treated in the above way are isolated from the rest with parchment insulators for 4–6 days – a sufficient time period during which the head of white cabbage loses its ability to perceive pollen from plants of its species. To obtain apomictic seeds, seeds were used at the initial stage of mass opening of flowers and during the next 8–10 days later. After removing the insulators, phenological observations were made on the growth of pods and seed embryos until the white cabbage seeds fully ripened.

The developed method of obtaining apomictic seed was tested on white head cabbage varieties during 2017–2021 years on experimental base of Institute of Vegetable and Melon NAAN. As objects of research used the reproductive plant of cabbage such cultivars as Yana, Lazurna, Lesia, Lika, Ukrainska osin, Kharkivska zimona, Bilosnizhka. As a result of the studies was found that double treatment of castrated buds by means of plant growth

regulators and alien pollen stimulated fully formation of apomictic seed for such varieties of white cabbage as Yana, Lesia, Lazurna and Lika. By the above means, we observed also incomplete formation of apomictic embryos (Table 1).

Table 1

The effect of growth regulators and alien pollen on the incidence of apomictic seed in different varieties of cabbage (data for 2017–2021)

Cultivar of cabbage	The number of buds involved in the experiment, samples	The pods formed as a result of the synergistic-effect of growth regulators and alien pollen on the initiation of buds growth, samples	The pods with underdeveloped apomictic seed, samples (%)	The number of underdeveloped apomictic seed, samples	The number of fully formed apomictic seed, samples
Lika	300	277	10 (3,61)	14	23
Lesia	194	176	6 (3,41)	8	17
Yana	244	221	11 (4,98)	15	4
Ukrainska osin	90	53	1 (1,89)	1	0
Bilosnizhka	243	222	10 (4,51)	14	0
Lazurna	256	231	13 (5,63)	17	2
Kharkivskaa zimova	293	266	16 (6,02)	20	0
Total:	1620	1446	67 (4,63)	89	46

The obtained experimental data show a significant influence genotype of cabbage varieties on used exogenous factors of growth unfertilized eggs. During the research had been received 46 samples of apomictic seed such cabbage cultivar as: Lika (23 seeds), Lesia (17 seeds), Yana (4 seeds) and Lazurna (2 seeds). Incomplete formation of apomictic seed was found in all investigated varietal genotypes of cabbage with frequency of 1,89–6,02 %.

Grown apomictic seeds from all investigated varietal genotypes of white cabbage had 100% similarity under the conditions of using potted seedlings. The obtained apomictic plants of each variety were characterized by the friendliness of maturation and did not have abnormal deviations in the process of organogenesis during the vegetative phase of development in comparison with the plants of the variety, which were propagated by the traditional method as a result of intravarietal free cross-pollination.

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