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HAPLOIDY USE WHEN CREATING RAPE SOURCE MATERIAL

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The article analyzes the literature on the importance of haploidy role in rape selection process. The results of researchers' investigations are given on this issue.

Selection is the cheapest, most effective and environmentally friendly method of crop production growth [2]. Selection plays a particularly important role according to current trends of increasing the energy value per unit of production and at the presence of problems having arisen because of dangerous pollution [8].

New selection objectives require full and objective information on existing source material used in selection and obtaining new forms, creation of which is provided by significant potential of species genotypic variability by features of adaptability and economic values [12].

Rape *(Brassica napus L.)* is one of leading industrial crops. In recent decades, owing to strong demand in the world market, areas of the crop in Ukraine have increased. This is due to increasing demand for vegetable oils as the main raw material for production of consumer goods [23].

Selection programs on rape of most countries are aimed at the following objectives:

- creation of high-yielding varieties of OO and OOO types and –O industrial type;
- improvement of the fatty acid composition of oil and protein meal quality;
- creation of rape hybrid based on cytoplasmic male sterility and incompatibility;
- resistance increase of varieties and hybrids to abiotic, biotic and edaphic factors of the environment;
- development of transgenic varieties and hybrids with agronomic characteristics [12, 23].

The introduction of biotechnology in genetic-selection process is becoming a key factor, which helps to find new promising areas of creating raw rape materials [5, 21, 27].

The most effective method of obtaining stable lines is a method of experimental haploidy [10, 27, 28]. Excluding multiple self-pollination of plants, it gives an opportunity to obtain homozygous lines from the material enriched with genetic ratio of material [20, 13, 14].

Haploidy is a result of embryo development with reduced (haploid) gametes from functionally equivalent cells by apomixes [6, 19]. Apomixes (Greek *απο* – out and *μιξις* – combination, mixing) is development of the embryo without fertilization [27].

In higher plants, there are several types of apomixes: parthenogenesis – the formation of the embryo from unfertilized eggs ; gynogenesis – development of the embryo from unfertilized eggs with sperm stimulated; apogamy – development of the embryo from synerhids or embryo sac antipodes; androgenesis – development of the embryo from pollen grains, or with sperm and oocyte nucleus during the last elimination; apospory – parthenogenetic or apohamic embryo development, combined with the formation of sexual generation in higher spore plants or embryo sac in angiosperms from somatic cells; accidental embryony – embryo formation from somatic cells of germ seed with its growing into embryo sac [28, 31, 43].

Haploidy is used to solve a number of genetic problems:

- detection of gene dosage effect;
- creation of aneuploids;
- research of quantitative traits genetics;
- genomic analysis, etc.

Androgesis is a special form of haploidy [27, 37].With the help of androgenesis it is possible to get a combination of the types of cytoplasm and genomes needed only in one or two generations [26].

Cultivation of pollen and anthers *in vitro* is the most promising method for obtaining haploids [29, 36, 41, 50]. Anther crop forms embryoids that develop in green seedlings [1, 3, 4, 33, 34].

The method of rape anther crop is based on the use of direct androgenesis effects *in vitro*. For the first time the use of anther crop methods for *Brassica* haploid genus was described by Cameo and Hinata in 1970. At present, the main factors that influence the induction of embryogenesis in anther *in vitro* are identified*:*

- growing conditions of donor plants;
- plants genotype;
- pollen development stage;
- pretreatment of buds;
- composition of the crop medium;
- anther cultivation conditions [38, 39, 46, 47, 49].

Increase of haploid number is observed when removing unfertilized seed germs from open flowers and during pollination of donor plants with irradiated pollen [30, 36, 44].

An important factor that can cause induction of embriodgenesis in the genus of *Brassica*, is temperature processing. Inducing symmetric divisions in microspores, thermal treatment increases the proportion of embryoids during their cultivation *in vitro*, whereas the effect of other physical factors that can increase the yield of embryos is inefficient [48].

Pretreatment of the source material is widely spread before entering explants in isolated crop, at reduced positive temperature of 4-6^{o}C, at which the inflorescence are placed in the refrigerator for 2-4 days [15, 16]. The positive impact of thermal shock on embriodgenesis induction in isolated anthers crops was confirmed by Keller, Armstrong, Casters, Schultz, Pauls, etc. [38, 39, 48].

Induction of sporophytic microspore or pollen grains cells development which leads to the development of embryoids or callus, and then plants regenerators depends on nutrient medium modifications by growth regulators [44]. These substances affect the differentiation and dedifferentiation of cells, initiate histogenesis, sharing and spreading of cells, or trigger growth and development of callus crops.

Research conducted by the Department of Biotechnology of SNU RSRIV showed that for induction of rape embroidgenesis and callus genesis, anthers should be planted into MS, NLN, B5 modified nutrient environment, which are the most successfully used in anther crops and microspores of other species of the genus *Brassica* [7, 9, 22, 32, 40, 42, 45]. On the basis of published data and work experience, the scheme has been developed and the research conducted on the content of growth regulators in the substrate [7, 9]. It is determined that embroidgenesis is actively held in a nutrient environment containing 6-BAP at a concentration of 4.0 mg/l, NOK – 0.3 mg/l. Induction of callus genesis is observed on environment containing growth regulators of auxins nature only – 2,4-D and NOK.

Scientists also claim that the considerable impact on morphogenesis is due to the concentration of sucrose as a source of carbohydrate supply and maintenance of osmotic pressure [3, 4]. It is noted that with increasing concentration of carbon, the number of embryos increases. The positive effect was observed in the crop environment with sucrose concentration 120 g/l.

According to A.A. Muravyov, an optimal nutrient substrate for spring rape anthers cultivation is modified Hamborha environment with addition of 2,4-D and NOK in concentration of 1.0 mg/l, iron chelates – 10.0 mg/l, mezoinozitis – 75.0 mg/l [15, 16]. 2,4-D at a concentration of 1.0 mg/l increases the frequency of induction of embryoids from 1.8% to 6.6%, and gibberellins acid at a concentration of 0.1 mg /l – from 0.9% to 7.4%. Androgenesis of spring rape is also affected by the origin and genotype of donor material. Usage of androgenic lines as donor plants increased embroidgenesis [15, 16].

The researchers also found that induction of the formation of androgenic rape structures is determined by balance between the concentration of exogenous 2,4-D in a modified crop medium and endogenous IOK content in anthers at the time of inoculation [9]. Such balance will be unique for each variety or hybrid. Selection of an optimal balance will manage the process of microspore morphogenesis in crops *in vitro* and contribute to acceleration of androgenic structures development.

The content of growth regulators of auxins nature in crop medium leads to the formation of embryoids of different development stages. They are distinguished by the morphology:

- a globular structure, matte color, up to 0.5-1.0 mm. When cultivated on organic environment, it forms callus from which individual plants are regenerated;
- poliembrioids of an oval shape, matte color that reach 1.5 mm. Formation of plant regenerators is observed;
- a heart-shaped bipolar structure, matte color, heart-shaped, up to 1.0 mm. It has a high proportion of plants regenerating;
- a torpedo-shaped bipolar structure, matte color. The shape resembles a torpedo up to 1.0 mm. Share of regeneration depends on the age of embryoids;
- a cotyledonary bipolar structure, matte color, resembles torpedo shape. It features enlarged cotyledons, which are fused to one another;
- an abnormal type of bipolar structure, matte color: without cotyledons or there are many of them. Plants regenerators are formed through secondary embryoid crop [24, 26, 29].

Getting a set number of haploid materials in crop *in vitro* makes it possible to use haploidy phenomenon not only in genetic research, but also in practical selection [11, 17, 18, 35].

Homozygous doubled haploids are used for rape hybrid development, stabilization of existing and creation of new varieties, and to identify unique genotypes arising from recombination of genes of parental forms [11, 15, 16, 35].

Conclusions. Thus, the use of haploid selection research opens up broad prospects. The most important thing is that haploidy is not just a special method of creating haploid forms, but it is closely linked to the selection at the polyploid level, effective use of mutagenic factors, the intensity of creating heterotic hybrids and transfer them to a sterile base, the ability to accelerate the selection of perennial plants and selection at a cellular level. Even this incomplete list of opportunities suggests that haploidy is one of the effective ways of genetics and plant selection.