GENETICS

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# GENERATION OF TRANSGENIC PLANTS FOR PRODUCTION OF OILS WITH IMPOTANT PHYSICOCHEMICAL PROPERTIES

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The production of seed oil is constantly growing, and the spheres of use are expanding, but food and technological quality of the seed oil produced now not always meet specific requirements of a balanced diet and of various industrial productions. Therefore, there is a need of improving the physical and chemical properties of oils, which are determined primarily by their fatty acid composition. The high content of unsaturated fatty acids provides prophylactic and therapeutic effects of seed oils, but it increases their ability to peroxidation. It is possible to distinguish two the most expedient ways of reducing the ability of seed oil to oxidize. The first way is connected with increasing the content and optimizing the ratio of basic forms of antioxidants, especially tocopherols. The second method is based on increasing the glyceride content of monounsaturated fatty acids, especially oleic acid. The increasing or decreasing (depending on the field of oil use) the content of glycerides of saturated fatty acids can be considered as the another effective direction of improving the fatty acid composition of oil. Selective-genetic improvement of content and quality of oil is considered as the most economical and ecologically safe method. The improvement of main physical and chemical properties of oil and its fatty acid composition can be reached iт two ways: by increasing in oil the content of some inherent components and by creating biogenous sources of oil with unusual for this culture composition. In terms of efficiency, transgenesis has obvious advantages to natural and induced mutagenesis. According to the conducted research, the use of transgenesis can be carried out on a broad set of oil plants. The most significant improvement of quality of oil can be expected in case of transfer to genotypes of plants–acceptors sections of DNA including the loci that control izoenzymatic composition and activity of elongase and desaturase of the major fatty acids. The systems of genetic regulation of fatty acid composition of oil for any oil-bearing crop cannot be recognized as completely established. Therefore, there is a need of carrying out its detailed genetic analysis on several cultures. It allows defining the best donors and acceptors of genetic structures for transgenesis. In view of mainly polygenic regulation of content and fatty acid composition of oil, it is necessary to define chromosomal localization of the loci that regulate them and to allocate the marker loci linked to them. The raw sources of oil should be standardized, and there should be models of oil with optimum composition taking into account directions of its use.

***Key words***: seed oil, fatty acid composition, glycerides of oleic acid, transgenesis, genetic analysis.

Seed oil is not only one of the leading components of a diet of a person, but it is also major raw source for food, pharmaceutical and a number of technical industries [2, 16, 34, 52]. Therefore, production of seed oil increases constantly, and spheres of industrial use – extend [61].

However, edible and technological qualities of the seed oil produced now not always meet specific requirements of a balanced diet and of various industrial productions.

Therefore there is a need to improve physical and chemical properties of seed oil which are defined first of all by their fatty acid composition [8]. Practical attention is noted to the possibility to create on the basis of seed oil such foodstuff and raw sources which would contain not only high technological properties, but also had a light effect on a human body and could be adapted at most to particular spheres of use.

It is known that fatty acids, which are parts of oil, distinguish by the length of a carbon chain and degree of its unsaturation, i.e. quantity and a relative position of diene bonds [40]. And the main difference between seed oil and adipose is in much higher rate of glycerides of unsaturated fatty acids in their composition, especially linoleic and linolenic acid. This feature is particularly denoted in oil plants which are grown on the territories with cool and moderate temperature condition. On the contrary, unsaturation of oil in cultures from tropical and subtropical zones is, as a rule, lower [50].

High content of unsaturated fatty acids provides useful effect of seed oil for prophylaxis and treatment of a wide range of diseases [14, 17]. However dominance of unsaturated fatty acids in the composition of seed oil can cause also negative physiological consequences as these components of oil are exposed to peroxidation and their intermediate products are extremely harmful for the health of people [30,40].

It is possible to distinguish two the most expedient ways to decline the ability of seed oil to oxidize.

The first way is to increase the number of natural antioxidants in oil, first of all, the number of tocopherols and to optimize a ratio of their main forms [14]. The advantage of this way is that tocopherols have also vitamin effect, which provides, at least, two important physiological functions – anti-sterile and antidystrophyc function [6, 35]. At the same time, there are also two circumstances that complicate an assessment of the practical importance of increase of oil resistance to oxidation with the use of effect of tocopherols. The first reason is in quite low total content of tocopherols even in the richest seed oil, the second reason relates to the probable pro-oxidant function of tocopherols.

The second way is to increase the content of glycerides of monounsaturated fatty acids in oil, especially oleic acid (C 18:1), which in resistance to peroxidation exceeds linoleic acid by 12 times (C 18:2) and linolenic acid by 25 times (C 18:3) [16].

Besides, oil with high rate of glycerides of oleic acid has a useful effect in relation to prevention or treatment of a wide range of diseases of a person, in particular, cardiovascular, oncologic, proctologic and probably diabetic diseases [42]. Oil with high content of an oleate has also good hydrodynamic properties and rather high thermal stability that provides possibilities to use it as a liquid lubricant, hydraulic liquid and cooking fats which are exposed to a heat treatment [6, 39, 46].

There are also some disadvantages of this way of quality improvement of oil. Physiological activity of oleic acid is smaller in comparison to linoleic acid. However according to some data, physiological activity of linoleic acid (activity of vitamin F) increases synergistically in the presence of an oleate. At the same time physiological activity of linolenic acid concedes to linoleic activity by nine times [5].

The increase of the content of saturated acids, first of all, of palmitic (C 16:0) and stearic acid (C 18:0) can be considered as the other effective way of improvement of fatty acid composition of oil.

Oil of this kind does not have such physiological activity of oil as that with high content of unsaturated acids, including oleic acid, but it surpasses the latter in resistance to peroxidation [9]. Besides, oil with the increased content of saturated acids has the highest heat stability [16].

The most effective spheres of use of oil with increased rate of glycerides of saturated fatty acids are the following: making of plastic technical lubricants, soaps, culinary oil that are adapted to high-temperature processing and production of margarine [6, 24, 27, 39]. The use of such oil in food industry is considered as undesirable because saturated fatty acids cause considerable increase of cholesterol in blood and enlarge the risk of cardiovascular diseases [24].

Thus, the following ways of improvement of fatty acid composition of seed oil can be distinguished: the increase of the content of glycerides of oleic acid, and also the increase or decrease (depending on the sphere of use of oil) the content of glycerides of saturated fatty acids.

However at the same time it is necessary to take into account that improvement of oil quality is the most effective at parallel increase of its content in marketable products which has also its independent value as seed oil has calories by two times more than proteins and carbohydrates [20]. Besides, getting of seed oil is carried out from primary renewable producer of energy which is autotrophic plants, and unsaturated fatty acids synthesized by olive plants are irreplaceable.

Although different families of oil plants significantly differ from each other in fatty acid composition of oil [27], more relevant approach to improve oil-bearing crops is creation of probably wider variety of this kind on the basis of one culture which is well adapted to an area of cultivation and that creates possibilities to get import-replaceable raw materials of multi-purpose use. At the same time the increased content of oil in any type of such products is desirable.

Selective-genetic improvement is considered as the most economic and ecologically safe method of improvement of content and quality of oil [60] as it has hereditary variability of these features [62].

The main methods of creation of genetic variety in content and quality of oil are natural [5] and induced [49] mutagenesis, and also transgenesis [33]. Each of these methods has some advantages and disadvantages.

The main disadvantage of use of a natural mutagenesis for extension of variety of oil-bearing crops in content and quality of oil is extremely small quantity of known mendelian loci or loci of the quantitative features with authentic effect in lipid composition of seeds. Oil-bearing crops are badly genetically mapped and among them corn is studied better than other crops. Therefore effectiveness of improvement of content and quality of oil in this culture with the use of its natural variety is rather high [23].

The main disadvantages of induced mutagenesis are its accidental, nondirectional character, low frequencies of getting mutations with the useful effect and simultaneous appearance of a series of mutations which do not have desirable changes of the chemical composition, but they initiate violation of physiological functions of a plant and often have the semi-lethal nature. Despite this fact, with the help of induced mutagenesis it became possible to expand considerably genetic variety of the main oil-bearing crops in fatty acid composition of oil, especially in sunflower [22, 42, 57], soy [44, 46, 47] and colza [31, 37,54].

The main disadvantages of a transgenesis are its technical complexity, unpredictable implications of introduction of foreign genetic structures to genomes of oil plants for biogenous sources of seed oil and for consumers of GMO – production. At the same time it is necessary to admit that in relation to effectiveness of creation of broad set of biogenous sources of seed oil with improved physical and chemical properties and for the solution of this task, transgenesis has obvious advantages to mutagenesis. Besides, unlike mutagenesis, transgenesis creates possibilities to make up genetic sources atypical for this culture, but that has industrially valuable fatty acid composition of oil and, as a result, has improved physical and chemical properties [1, 8, 36, 59].

According to the conducted research, the use of transgenesis can be carried out on a broad set of oil plants and can be very effective [8, 21, 27, 29, 51].

Basis for creation of useful genetic variety in content and quality of oil by method of transgenesis is to define mechanisms of synthesis of lipids, in particular, of glycerides and systems of its genetic regulation.

Within the modern studies [4, 17, 45, 52] the process of synthesis of lipids in superior plants can be divided conditionally into three stages. The first of them is a set of reactions of transformations of sucrose into precursors of fatty acids. The second stage is a set of reactions of transmutations of the fatty acids catalyzed by two classes of enzymes – elongases (synthases), that extend a carbonic chain of acid and desaturases, that turn saturated bonds into unsaturated. Finally, the third stage is an etherification of generated fatty acids by glycerin. At the same time it is necessary to mention that reactions of the elongation of fatty acids are carried out with an involvement of Acetyl-KoA and acyl which transport proteins. The diagram of synthesis of fatty acids in the superior plants is given in Fig. 1.

It is discovered that each reaction of formation of fatty acids is catalyzed by specific enzyme which activity is controlled by a separate gene or a cluster of genes [41]. At the same time fatty acid composition of oil in all main oil-bearing crops has quantitative genetic nature. It is regulated by several nonallelic genetic factors and their effect is modified by polygenic loci of the quantitative features [12, 25, 32, 56].

However there are no doubts that in case of extension of the useful genetic variety in fatty acid composition of oil using the method of transgenesis the most significant improvement of quality of oil can be expected in case of transfer to genotypes of plants – acceptors of sections of DNA including the loci that control izoenzymatic composition and activity of elongase and desaturase of the major fatty acids which provide the improvement of the physical and chemical properties of seed oil and its practical value at nutrition and in the industrial use [10, 26].

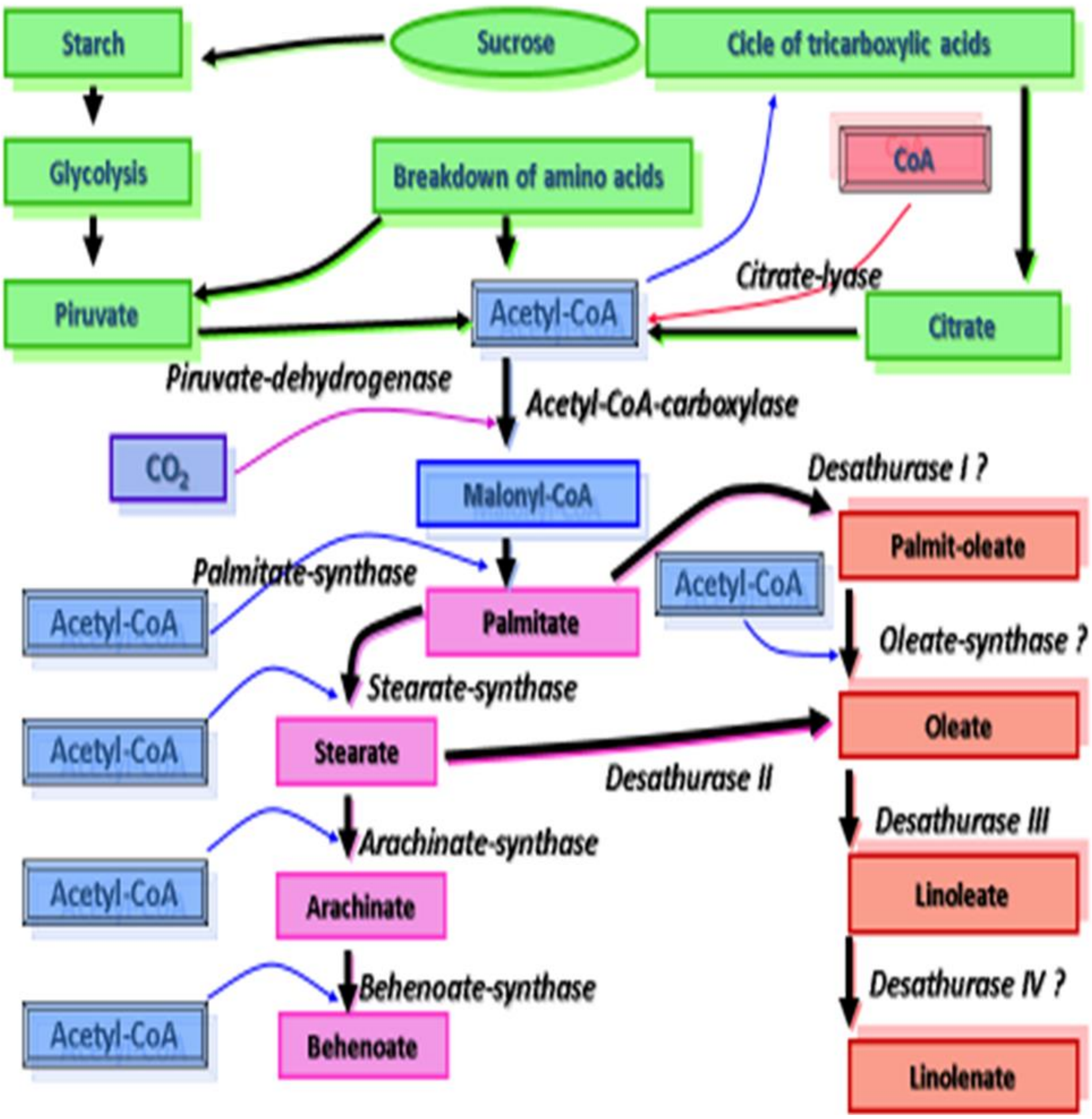


*Fig. 1.* Principled scheme of fatty acids synthesis in higher plants (Tymchuk, Kharchenko. 2020)

Technologies of getting of transgene plants, despite the complexity, are developed rather well and in the presence of the corresponding equipment, there are no restrictions for improvement of oil-bearing crops in content and quality of oil. Although some operations of this process can be apparently optimized. At the same time, there is a number of unsolved problems, which significantly affect effectiveness of transgene improvement of oil-bearing crops.

There is a need to make up interrelations between main physical and chemical properties of oil and its fatty acid composition. Its improvement can be reached by two ways: to increase in oil the content of some inherent components [55] and to create biogenous sources of oil with unusual for this culture composition [1, 36, 59]. It is quite difficult to define which way is preferable; however it is obvious that raw sources of oil should be standardized, and there should be models of oil with optimum composition.

The systems of genetic regulation of fatty acid composition of oil for any oil-bearing crop cannot be recognized as completely established. Therefore, there is a need of carrying out its detailed genetic analysis on one and several cultures, depending on technical capabilities. It allows defining the best donors and acceptors of genetic structures for transgenesis.

In view of mainly polygenic regulation of content and fatty acid composition of oil, it is necessary to define chromosomal localization of the loci that regulate them, to allocate the marker loci linked to them and to establish the most expressional sites of chromosomes that regulate these features. These particular targeted and marked sites of DNA (not every fragment of DNA of the donor) at transfer will lead to the greatest effectiveness of transgenesis.

There is a need of the analysis of the structural and functional compatibility of the transfer at transgenesis from the donor of the site of DNA with DNA of a plasmid and an assessment of phenotypic expressivity of the modified plasmid in an acceptor-plant.

The assessment of physiological consequences of transgene modification of content and quality of oil from biogenous plant source and the analysis of ecological consequences of cultivation of transgene plants is relevant for the study.

According to preliminary data these problems, in a view of the analysis of the latest special literature, have scientific novelty and practical importance.

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## СТВОРЕННЯ ТРАНСГЕННИХ РОСЛИН ДЛЯ ВИРОБНИЦТВА ОЛІЙ З ВАЖЛИВИМИ ФІЗИКО-ХІМІЧНИМИ ВЛАСТИВОСТЯМИ

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Виробництво насіннєвої олії постійно зростає, сфери використання – розширюються, проте її харчової та технологічної якості не завжди відповідають специфічним вимогам збалансованих дієт та промислових виробництв. Тому існує необхідність удосконалення фізичних та хімічних властивостей насіннєвих олій, які визначаються, насамперед, їх жирнокислотним складом. Високий вміст ненасичених жирних кислот забезпечує профілактичну та лікувальну дію олій, але пов'язаний водночас з нестійкістю до перекисного окиснення. Можливо виділити два найбільш доцільних методи зниження здатності олій до окиснення. Перший полягає у збільшенні вмісту в олії природніх антиоксидантів, насамперед, токоферолів, та оптимізації співвідношення їх основних форм, другий – у підвищенні вмісту у оліях гліцеридів мононенасичених жирних кислот, особливо олеїнової кислоти. Доцільне також використання іншого напрямку удосконалення жирнокислотного складу насіннєвих олій шляхом збільшення або зменшення (в залежності від сфери використання олії) вмісту гліцеридів насичених жирних кислот.Селекційно-генетичні методи підвищення вмісту та поліпшення якості олій вважаються найбільш економічно вигідними та екологічно безпечними. Порівняння зі штучним та спонтанним мутагенезом надає певну перевагу трансгенезу у створенні біогенних джерел поліпшених насіннєвих олій. Згідно з проведеним дослідженням, трансгенез може виявити значну ефективність у широкого спектру олійних культур, при цьому найбільш вагоме підвищення якості олії очікується у разі перенесення до генотипу рослини–акцептора локусу ДНК, що контролює ізоферментний склад та активність елонгази та десатурази основних жирних кислот. Системи генетичної регуляції жирнокислотного складу олій повністю не з'ясовані, що вказує на необхідність проведення детального генетичного аналізу з метою виявлення донорів та акцепторів генетичних структур для трансгенезу. Оскільки вміст та жирнокислотний склад олій контролюються головним чином полігенно, доцільно визначити хромосомну локалізацію генів, що їх контролюють, та виділити зчеплені з ними маркерні локуси.Доцільне введення стандартизації сировинних джерел олії та створення моделей олії з оптимальним складом із врахуванням напрямків її використання.

**Ключові слова**: олія насіння, жирнокислотний склад олії, гліцериди олеїнової кислоти, трансгенез, генетичний аналіз

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