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Genetics and Selection

Mutations in the Seeds of *Actinidia kolomikta* Induced by Nitrosomethyl Urea and Nitrosoethyl Urea

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ABSTRACT. Experiments revealed that germination ability of the seeds of *Actinidia kolomikta* reduces to some degree and the spectrum of morphological mutations in seeds increases as a result of the action of supermutagens nitrosomethyl urea (NMU) and nitrosoethyl urea (NEU). *© 2009 Bull. Georg. Natl. Acad. Sci.*

Key words: *mutagen, nitrosomethyl urea, nitrosoethyl urea, Actinidia kolomikta, mutations.*

Mutagenesis is a process of generation of hereditary changes (mutations) which occur naturally (spontaneous mutagenesis) or artificially (induced mutagenesis) under the influence of various physical and chemical agents – mutagens. Mutation is a change of the genetic information of an organism, i.e. a change in the DNA molecules followed by the changes of some traits of an organism. The process is revertible and the changed traits are hereditary. Induced mutagenesis enables to obtain mutant plants which may be used in further selection works to get initial forms of new plant varieties [1, 2].

Significant progress in the development of efficient methods for chemical mutagenesis was made (since 1966) after I.A. Rapoport discovered superpower mutagen agents and named them supermutagens. Those increase mutation frequency by 1,000 and 10,000 times. Nitrosomethyl urea and nitrosoethyl urea belong to such kind of supermutagens [3].

According to induced useful mutations in agricultural plants, supermutagens, by their genetic effectiveness, exceed mutagen agents known earlier. Rapoport has studied the effectiveness of chemical mutagens at all levels of the genome organization.

Nitrosomethyl urea (NMU) and nitrosomethyl urea (NEU) supermutagens are used in selection to get induced mutation. In small concentrations they are used as growth stimulators. In plant selection, while induced mutation takes place, nonhereditary changes are met besides hereditary changes. Those are phenocopies and morphoses which are preserved for several generations. Such changes are called long-term modification.

The above mentioned supermutagens have an ability to enter the cell and cause such reconstruction of the genetic material when gene mutations are generated primarily and chromosome aberrations are insignificant. They frequently cause systemic mutations which often serve as a basis for taxonomic differentiation.

It is believed that the high genetic ability of supermutagens is caused by the sum action of such compounds on the DNA molecules. Interaction of NMU and NEU with DNA molecules, followed by regrouping of pairs of nitrogenous bases, may occur in both ways: transition and transversion [3].

It is observed that supermutagens, such as nitrosomethyl urea and nitrosoethyl urea, induce more useful mutations in agricultural plants than the known earlier mutagen agents.

In the mutation processes caused by mutagenesis a general regularity is revealed: chemical mutagens affect nucleoproteins, genes and generate new traits. At the same time a large spectrum of mutagen changes is observed.

Literary data confirm that supermutagens are used effectively to obtain new forms. Besides, seeds, treated with them, are distinguished for high vitality. Chemical mutagens closely react with DNA but never become their component part.

The subject of our research is *Actinidia kolomikta*

Actinidia kolomikta is a species of deciduous dioecious woody vines in the genus *Actinidia* native to temperate mixed forests of the Russian Far East, Korea, Japan and China (Eastern Asiatic Region). It ultimately grows to 8-15 m. Actinidiaceae, is a small family of plants. It includes three genera: *Actinidia kolomikta, Actinidia argutta and Actinidia polygama,* and about 360 species. Among them *A. kolomikta* is the hardiest species in the genus, at least down to about -40°C [4].

A. kolomikta is tolerant of varying soil conditions, including infertile soil. Good drainage and an adequate moisture supply are needed. This vine can be grown both for its beautiful foliage and its fruit. Its vigorous growth habit makes it suitable for a variety of cover-type uses such as trellises, fences, or walls.

The stem of *A. kolomikta* is thin, smooth, branching and covered with dark-brown scaly bark. The leaves are alternate, simple, with a dentate margin and a long petiole. They are large, with the length of 15 cm and the width of 10 cm. Young leaves of male plant have green, white, pink, rose, or red variegation when grown in good light. In plants that are of bearing age, the outer half of the leaf turns color when it is time to flower.

The flowers are dioecious, pure white or slightly pinkish. There are 1-3 flowers with the diameter of 20 mm in the female inflorescence, and 12-17 ones with the diameter of 10-15 mm in the male. Actinidia blooms in May-June.

The fruit is a large berry containing numerous small seeds; in most species the fruit is edible. It is of cylindrical form, soft, 20-30 mm in length and 8-15 mm in diameter with smooth peel. Fruit is dark green with longitudinal stripes. Peduncle is elongated. In this they resemble the gooseberry. Hence its other name - Amur gooseberries.

Fruit of *A. kolomikta* is rich in vitamins C and E, serotonin and potassium and is purported to have antioxidant activity, and is low in fat with no cholesterol.

Actinidia is well reproduced by seeds and vegetatively. Before sowing the seeds require stratification for 45-60 days. The best period to sow is spring and autumn. Vegetative breeding is effected by lignified or green cuttings [4].

In order to get new selection material for *A. kolomikta* we studied the mutagenic action of the chemical mutagens NEU and NMU on seeds. It was established for various subtropical plants (feijoa, loquat, citrus) that the mentioned mutagens have a fairly effective influence in hereditary mutations.

Material and methods. For the experiment we were guided by the method worked out by N.Zozii and S.Makarova at the Moscow Institute of Chemical Physics [5]. The method has been modified by Sh. Goliadze, I. Kerkadze and A. Diasamidze for citrus plants at the department of genetics and selection of the Scientific-Research Institute of Tea and Subtropical Cultures in Anaseuli, Georgia [6, 7].

Material for experiments was taken on the breeding site of the Chakvi Scientific Centre at the Scientific-Research Institute of Tea and Subtropical Cultures. Seeds of *Actinidia*, after being dried at room temperature for 3-4 days, were subjected to 24-hour exposure with different concentrations of NMU and NEU under laboratory conditions. The experiment was carried out with unpeeled seeds of *Actinidia kolomikta* because they are fine enough and it is practically impossible to peel them. It should be noted that dense peel is resistant to the mutagen matter penetration into the kernel.

Treated seeds were sowed in vegetation boxes in hot-houses. We have calculated the germination percentage and determined the spectrum of the changes that occured (Tables 1, 2).

As seen from the Tables, the seed germination percentage is low because of the impact of the solution concentration. In particular, relatively high concentration of the solution inhibits the germination ability of seeds. In some cases the effect may even be lethal.

One of the effects of exposure to chemical mutagens is the origin of the so-called albino plants. This fact may be considered as a result of inhibiting influence of mutagens on those genes of plants which are responsible for the generation and functioning of chlorophyll grains. Formation of such albino plants in larger quantity occurs by using strong solutions.

The effect of chemical mutagen emphasized the phenomenon of polyembryony of seeds. The polyembryony phenomenon is not often met in plants. In the plants under study such seeds practically do not occur or if they do, in very small percentage. The effect of small doses of mutagens NMU and MEU is to stimulate germination of additional embryos in the nucellus.

Table 1

Changes in seeds of *Actinidia kolomikta* caused by the effect of mutagen NMU

NN	Mutagen concentration	Number of seeds treated with the mutagen	Percentage of germination	Percentage of albinism	Percentage of polyembryony	Total amount of mutations	Percentage of mutations
1	1:1500	100	85.0	0	1.8	20	23.5
2	1:1000	100	78.0	0	3.4	25	32.0
3	1:500	100	55.0.	2	6.9	30	54.5
Control		100	98.0	0	1.0	2.0	2.0

Table 2

Changes in seeds of *Actinidia kolomikta* caused by the effect of mutagen NEU

NN	Mutagen concentration	Number of seeds treated with the mutagen	Percentage of germination	Percentage of albinism	Percentage of polyembryony	Total amount of mutations	Percentage of mutations
1	1:1500	100	84.0	0	1.6	20	23.8
2	1:1000	100	75.0	0	2.5	23	30.6
3	1:500	100	62.0.	5.1	7.7	32	51.6
Control		100	98.0	0	1.0	2.0	2.0

The aim of using chemical mutagen is to increase the spectrum of changed traits of plants (given in Tables 1, 2). The changes involve plant's height, leaf's contour, crenation, hairiness, location, etc.

Tables 1 and 2 show that the total amount of mutations increases in a certain degree with the increase of mutagen concentration. The majority of mutations are negative. For example, albino plants, such as those with misshapen leaves, crooked stems, fascial off-shoots, downiness, different growth intensity, etc., die at the age of 2-3 months.

So long as the plants are at present in the juvenile phase of development the mutation spectrum is not revealed fully. It will be observed better after the plant enters the fruit bearing phase. It should be also noted that some morphological traits are of epigenetic nature and are eliminated in new shoots in the same year or in the second vegetation period. Fascial forms with irregular distribution of undeveloped leaves belong to stable type of mutations. How promising the mentioned forms are will be evaluated after fruit-bearing.

გენეტიკა და სელექცია

Actinidia kolomikta-ს თესლებზე სუპერმუტაგენების - ნიტროზომეთილშარდოვანას და ნიტროზოეთილშარდოვანას ზემოქმედებით გამოწვეული ცვლილებები

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აქტინიდია კოლომიქტას ახალი სელექციური მასალის მიღების მიზნით შესწავლილ იქნა ქიმიური მუტაგენების ნიტროზოეთილშარდოვანას (ნეშ) და ნიტროზომეთილშარდოვანას (ნმშ) მუტაგენური ზეგავლენა თესლებზე.

დამუშავებულ თესლებს აღმოსაცენებლად ვთესავდით სავეგეტაციო ყუთებში, ყუთებს ვათავსებდით სათბურებში. ვითვლიდით აღმოცენების პროცენტულ მაჩვენებელს და აღვრიცხავდით წარმოქმნილი ცვალებადობის სპექტრს.

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