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DETERMINATION OF THE LEVEL OF ANDROGENESIS IN TOBACCO

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ABSTRACT

Androgenesis is the newest and most secure method to obtain haploid plants *in vitro*, where vegetative or generative nucleus of a pollen grain is stimulated to develop into a haploid individual. There are different possibilities for regeneration and formation of microspores in various genotypes of tobacco. In this case, the level of androgenesis was investigated in three tobacco genotypes. NN-media was used as a basic medium for microspores development and MS-media for rhizogenesis and organogenesis, together with adequate combinations of plant hormones (JAA, BAP, adenine, glutamine and kinetine).

Key words: haploids, tobacco, androgenesis, medium

ОДРЕДУВАЊЕ НА СТЕПЕНОТ НА АНДРОГЕНЕЗА КАЈ ТУТУНОТ

Методата на андрогенеза претставува најнова и најсигурна метода за добивање на хаплоидни растенија in vitro, каде вегетативното или генеративното јадро од поленовото зрно се стимулира за добивање на хаплоидни единки. Можноста за регенерација и формирање намикроспори кај одредени генотипови од тутунските растенија се различни, за таа цел во овој труд вршевме испитувње на степенот на андрогенеза кај три различни генотипови тутун. Како основен медиум за развиток на микроспорите беше користен NN- медиумот (Nitch J. P. и Nitch C., 1969), додека за ризогенеза и органогенеза MS-медиум(Murashige T., и Skoog F., 1962), како и соодветни комбинациина растителни хормони и тоа: ЈАА, БАП, аденин, глутамин и кинетин.

Клучни зборови: андрогенеза, медиум, генотипови, тутун

INTRODUCTION

Haploid plants can be obtained by isolation of anthers *in vitro* in two ways:

- direct , with formation of embryoids from the pollen grain (microspore), and

- indirect, with callus development and formation of haploid embryoids or adventive buds [6].

The latter type of development is unsuitable, because callus as a starting material is of heterogenic nature (haploids and diploids).

Tobacco is an ideal plant for obtaining haploid cultures in direct way. Tobacco cultures produce an explosion of haploids, which are now used in hybridization processes. Some authors stimulated the production of female gametes (*gynogenesis*) or male gametes (*androgenesis*) in direct haploid individuals. They came to conclusion that in gynogenesis, which is carried out *in vivo*, female cells are stimulated to grow without fertilization. In androgenesis, which is carried out only *in vitro*, vegetative or generative nuclei from pollen grains are stimulated to develop haploid plants without fertilization. The literature on androgenesis *in vitro* [6, 1] clearly shows that the Solanaceae family species are capable of regeneration of haploids from isolated anthers.

The goal of this paper was to investigate the genetic potential of some newly created lines in vitro, using the method of androgenesis.

MATERIALS AND METHODS

Anthers from three oriental tobacco lines were used for determination of the level of androgenesis: Line 137, Line 147 and Line 208. We used Nitsch-Nitsch (NN),[5] as a basal medium, and Murashige-Skoog (MS), [4] medium was used for rhizogenesis and organogenesis, together wit adequate combinations of plant hormones like JAA, BAR, adenine, glutamine and kinetin. Sterilization of buds was made with 2% HgCl and 70% alcohol, and they were finally washed in sterilized water. Androgenetic potential was evaluated by the classification of Mityko and Fari [3]:

- poor androgenetic potential - up to 5% embryogenetic anthers
- average androgenetic potential - 5 - 10% embryogenetic anthers
- good androgenetic potential - 15-30% embryogenetic anthers
- high androgenetic potential - over 30% embryogenetic anthers

RESULTS AND DISCUSION

Androgenesis can be induced in many agricultural plants, but the ability of some species for successful microspores propagation is often limited and depends on the genotype itself, i.e. on the variety. The choice of treatment that should be applied at *in vitro* conditions is based on the immense literature data on anthers and their regeneration [2], paying equal attention to the specificity of each genotype for regeneration in practice.

According to the results of induction of haploid embryos from anthers of the investigated tobacco lines (Table 1), line L.132 has the best embryogenetic potential and the highest percentage of anthers (32%) from those set up for regeneration.

Table 1 . Induction of haploid embryos from anthers in tobacco

Lines	Number of anthers	Embryogenetic anthers %	Callus anthers %	Embryogenetic potential
Line 137 F2	40 ± 4	32 ± 2,1	5 ± 1	High
Line 147 F2	36 ± 2	24 ± 3,0	2 ± 2	Good
Line 208 F3	40 ± 1	24 ± 2,5	3 ± 1,5	Good

Table 2. Morphological properties of haploid embryos from anthers in tobacco

Lines	Number of haploid plants	Plant height, cm	Root length
Line 137 F2	55 ± 0,5	3,4 ± 0,5	1,2 ± 0,4
Line 147 F2	24 ± 1,2	5,0 ± 0,7	1,0 ± 0,9
Line 208 F3	34 ± 2,0	4,8 ± 1,2	1,5 ± 1,5

Fig.1 Introduction of haploid emryos anther in tobacco

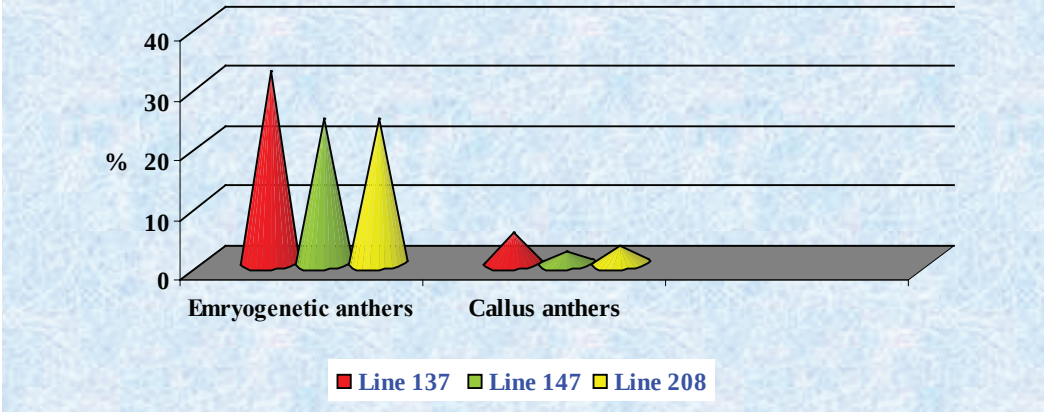
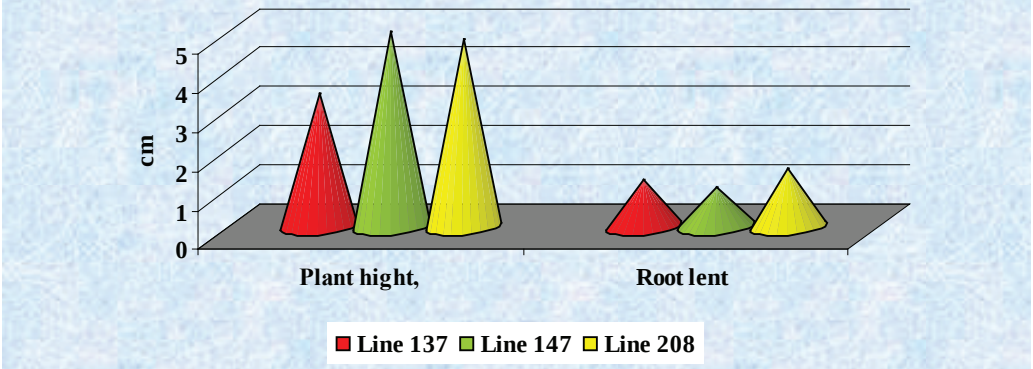


Fig. 2. Morphological properties of haploid embryos from anthers in tobacco



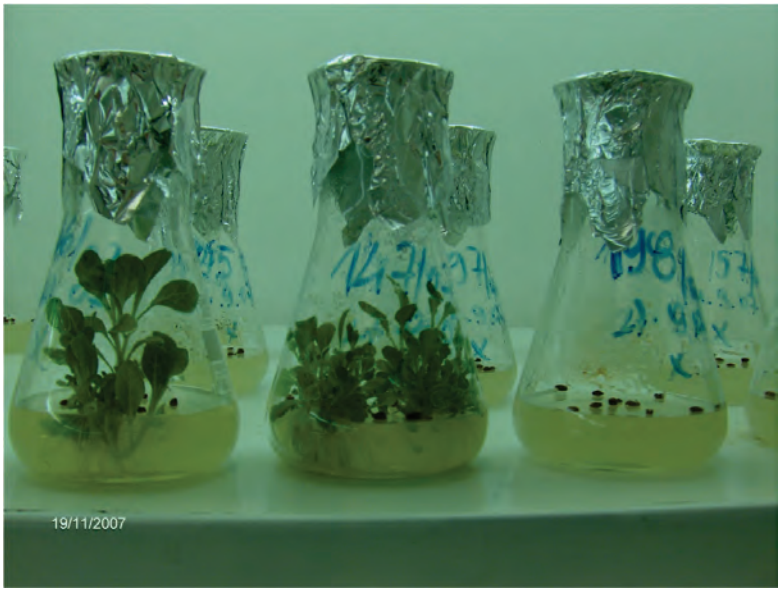


Photo 1. Haploid tobacco plants

Referring to morphological characters of the haploid embryos (Table 2), it can be stated that L. 137 has the highest number of haploid plants, which confirms that it possesses the best embryogenetic potential among all other tobacco lines investigated, according to the classification of Mityko & Fari [3].

CONCLUSION

According to the results, genotypes included in investigations have different abilities for embryoids formation and the callus formation in all of them is minimum.

- The greatest genetic potential was noted in the line L 137 (32%), which yielded the highest number of haploid plants (55).

- According to the classification of Mityko and Fari [3], the androgenetic potential is good in lines L 147 and L 208 and high in L 137.

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