

Determination of the level of androgenesis in tobacco (*Nicotiana tabacum* L.)

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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 methods 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-medium was used as a basic medium for microspores development and MS-medium for rhizogenesis and organogenesis, together with adequate combinations of plant hormones (JAA, BAP, adenine, glutamine and kinetine).

KEYWORDS: haploids, tobacco, androgenesis, medium

INTRODUCTION

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

Directly, with formation of embryoids from the pollen grain (microspore), and indirectly, with callus development and formation of haploid embryoids or adventive buds [7].

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 haploid individuals produced directly. 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* [7, 1] clearly shows that species from the Solanaceae family are capable for 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 (Line 137, Line 147 and Line 208) were used for determination of the level of androgenesis. We used Nitsch-Nitsch [5], abbreviated as (NN), as a basal medium, and a Murashige-Skoog [4] medium (MS), was used for rhizogenesis and organogenesis, with adequate combinations of plant hormones of JAA, BAR, adenine, glutamine and kinetin, respectively. 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 DISCUSSION

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 reaction of the genotype. The choice of the treatment that should be applied at *in vitro* conditions is based on the immense literature data on anther cultures 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%) among those set up for regeneration.

Table 1 . Induction of haploid embryos from anthers in tobacco (*Nicotiana tabacum*)

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

Referring to the morphological characters of the haploid embryos (Table 2), it can be stated that L. 137 has the highest number of haploid plants (55), which confirms that it possesses the best embryogenetic potential among the three tobacco lines investigated, according to the classification of Mityko & Fari [3].

Table 2. Morphological properties of haploid plants from anthers in tobacco (*Nicotiana tabacum*)

Lines	Number of haploid plants	Plant height, cm	Root length, cm
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

CONCLUSION

According to the results, genotypes included in investigations have different abilities for embryoid formation and the callus formation in all of them proved to be minimal. 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 was found as good in lines L 147 and L 208, and high in L 137.

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Photo 1. Haploid tobacco plants