Protein spectra and phenotype evaluation of two Macedonian tobacco local varieties

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Abstract

Two local tobacco varieties Prilep 65/94 Prilep and 79/94 are evaluated. Their phenotypic characterisation is presented by plant height, number of leaves, leaf size, duration of growing period. Yield ranges in production field conditions are reported

Polyacrylamide gel electrophoresis is used to observe the genetic similarity/difference between both local varieties.. The separation of alcohol-soluble proteins of tobacco seeds is carried out in 12% polyacrylamide gels with acetate buffer (pH=3.1). The electrophoregrams show very close genetic similarity of examined local varieties

Introduction

Variety is the basic factor for obtaining a standard and good quality tobacco of a certain type, which will be recognizable on the market

In the scope of type Prilep, 15 varieties have been approved and registered. Each of them is distinguished by some specific characteristics, but in the same time they are typical for the type Prilep.

To obtain a specific tobacco raw, the varieties should be grown in adequate soils, with application of all necessary cultural practices.

Among the varieties of Prilep tobacco, the new creations Prilep 65/94 and Prilep 79/94 are distinguished by their quality and provoke an increasing interest for their use in mass production. However, for continuous exchange of the breeding material, its collecting and storing in gene-bank, beside analyses of the phenotype characteristics, it is necessary to make identification by the method of electrophoresis, to protect the authorship and to prevent the variety from plagiarism. This method enables separation of protein molecules on the basis of their different motion in an electric field.

Seed storage proteins as genetic markers have been used to assess variation in cereal populations, landraces and cultivars (Drapper, 1987; Gepts, 1995). As these proteins are direct gene products, relatively free of environmental effects, they can provide either independently or in addition to other analyses, a reasonably accurate measure of genetic diversity. Recently it was presented that seed proteins could be used for comparison between tobacco varieties (Deng et al, 1998). However little is known about the practical application of proper electrophoretic techniques for of tobacco varieties identification.

This study is aimed to discuss the application of seed protein electrophoresis for tobacco variety identification when is carried out a parallel field evaluation.

Materials and methods

Polyacrylamide gel electrophoresis is used to observe the genetic similarity/difference between both local varieties: Prilep 79/94 and Prilep 65/94. The separation of alcohol soluble protein fraction is carried out in 12% polyacrylamide gels with acetate buffer (pH=3.1) using a

vertical slab gel unit (1mm thin gel). Tobacco seeds (70mg) are ground to a fine powder and extracted with 5M urea/50% alcohol solution over a night. than centrifuged at 16 000g for 20 minutes. 20-40 µl per slot are applied for each sample. Protein patters are developed after staining with Coomasie Briliant Blue R250 and TCA-acid solution (Stoyanova, 1991).

Results and discussion

Morphological, biological and productional characteristics

The variety Prilep 65/94 was registered in 1999. It achieves higher yield and better quality compared to the standard P 12-2/1. The plants are ellipsoid and cone-shaped. The average height with inflorescence is 60-65 cm. The average number of leaves is about 50.

The color of leaves and stalk in this variety is light green, which distinguishes it from the other varieties of the type Prilep. The size of the largest leaf is 21.02 cm in length and 12.3 cm in width. The flower bud is semi-round and dense. The capsule has an ovoid form.

The variety Prilep 65/94 is suitable for loose, warm, light and porous soils, not so rich with nutrients in watering conditions, but it gives good results in medium rich soils, without irrigation. The spacing is 40-45 cm between rows and 12-15 cm in the row.

The length of the growing period from transplanting to the beginning of flowering is 60 days, and to 50 % of flowering 70 days. The average yield ranges 1450 - 2600 kg/ha. It is resistant to damping off and bassara, and tolerant to blue mould.

The color of the cured leaves from the middle belt is gold yellow and that from the upper belt is light orange. Leaves have fine tissue, with high substantiality. The raw of this variety is typical for the type Prilep. It has a pleasant aroma and a very good taste during degustation, without irritation in smoking. Analyses based on the regulations for purchase and grading of oriental tobaccos show that it has a high presence of I and II grade.

Figure1 - Prilep 65/94

The variety Prilep 79/94 was registered in 1999.The average plant height with inflorescence is 60-70 cm. Plant habitus is elliptical and cone-shaped. The number of leaves per plant is over 50. The size of the largest leaf is 20 cm in length and 12.7 cm in width. Flower bud is round and dense. The form of the capsule is usually ovoid. The variety is suitable for loose and warm soils, with medium supply of nutrients; it gives better results in conditions of irrigation. Transplanting density is 40-45 cm between rows and 12-15 cm in the row. Duration of the growing period is 63 days from transplanting to the beginning of flowering, i.e. 72 days to 50% of flowering.

is resistant to bassara and tolerant to viruses. Prilep 79/94 belongs to the group of small-leaf oriental tobaccos; its aromatics is very close to that of the standard variety P12-2/1. The tissue of cured leaf is fine, subtle, with high substantiality. The color of the middle leaves is light orange and that of the upper leaves The average yield in productional conditions ranges 1450 - 2500 kg/ha.

It light red. The presence of high grades (I, II and III) is very high and in normal conditions it exceeds 70%.

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Figure 2 - Prilep 79/94

Evaluation by electrophoretic separation of proteins

The electrophoregrams of the both local varieties are very similar (Fig.3). They are composed by equal number of well developed protein components. As presented in the figure three groups of components are distinguished according to their relative mobility in the gel: A, B and C. Small differences by the density of some components are observed between evaluated varieties . More clear the differences appears between the components in groups A and B. Although the discussed differences there should be pointed that the electrophoretic patterns of both tobacco varieties (Prilep 79/94 and Prilep 65/94) are very close. The electrophoresis of seed proteins show very close genetic similarity of examined local varieties.

Conclusions

Polyacrilamide gel electrophoresis of seed proteins could be used for tobacco variety identification. The electrophoregrams of local varieties Prilep 65/94 Prilep and 79/94 show very close genetic similarity between them.

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