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COMPARATIVE STUDY OF DROUGHT TOLERANCE OF VIRGINIA TOBACCO GENOTYPES DIFFERING IN ORIGIN AND THEIR CORRESPONDING HYBRID PROGENIES

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Abstract

Soil drought is a serious problem in agriculture leading to considerable loss of production. Tobacco is relatively sensitive to water scarcity, and the selection of resistant drought varieties is of particular importance. In this study we compared the tolerance to three different levels of drought of six genotypes of Virginia tobacco – two varieties were introduced in Bulgaria, two lines are of Bulgarian origin, and two are their hybrids. Biochemical markers of stress (malondialdehyde, hydrogen peroxide and free proline) were determined. The results showed that the Polish genotype V 385 and one of the Bulgarian lines L 0842 were moderately sensitive to drought, while the parental genotypes – the American variety Coker 254 and the Bulgarian line L 0543, as well as the two newly created Bulgarian hybrids H 27 and H 135 were relatively resistant to drought. These data can serve as a basis for selection and development of new genotypes with increased resistance to drought.

Key words: *Nicotiana tabacum* L., stress markers, water deficiency

Introduction. Abiotic stresses cause significant losses to agricultural production worldwide. Drought is one of the main problems in agriculture, as this unfavourable environmental factor hinders plants from realizing their genetic potential [1]. Drought induces various physiological, biochemical and molecular responses in plants. Water deficit retards tobacco growth, reduces plant height and

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leaf area and disrupts key physiological processes [2]. Plant responses to drought vary significantly depending on the duration of the stress, the plant species, and the stage of plant development [3].

Virginia (flue-cured) tobacco originates from the subtropics and is one of the most important commercial crops in the world, and is quite sensitive to water deficit during the early ontogenic stages like leaf growth [4]. Studies by Maw et al. [5] found that drought was most detrimental for tobacco growth between weeks 8 and 10, but the effect was most pernicious in the sixth and seventh weeks after plants transplantation. These periods coincide with the periods of rapid growth of leaves and stems.

The lack of rainfall and irrigation under field conditions can be managed by breeding of drought-tolerant varieties [6]. When one genotype has better productivity than another under conditions of severe drought, it is relatively more drought resistant [7]. Establishing plant response mechanisms related to drought tolerance is an essential part of creating stress-tolerant crops [8]. The aim of the study was to assess the tolerance to drought of Virginia tobacco genotypes of different geographic origin and their hybrids for future inclusion in breeding programmes to create new drought-tolerant cultivars.

Materials and methods. ***Plant material and treatments.*** We studied the tolerance to drought of flue-cured Virginia tobacco (*Nicotiana tabacum* L.) plants cv. Coker 254 (American origin), Virginia 385 (Polish origin) and their hybrid H 27 (Bulgarian origin), and two Bulgarian lines (L 0543 and L 0842) and their hybrid H 135. Parental genotypes Coker 254, Virginia 385, L 0543, and L 0842 were described as tolerant to diseases caused by Potyvirus and/or Tobamovirus [9]. Our preliminary investigations showed that both hybrids also have a potential to be virus resistant.

Seeds of all six genotypes were germinated in a growth chamber (Float System for Producing Tobacco Seedlings), then the seedlings were grown as soil culture in growth chamber under controlled conditions – 16/8 h photoperiod, light density 150 µmol m^{-2} s^{-1}, temperature 25 °/18 °C day/night and 75% relative air humidity. Plants at the 4th leaf stage were transplanted to individual pots (65/65/60 mm). Five uniform plants of each genotype at the 6th true leaf stage were subjected to water deficit treatment for 4 weeks. Water stress was conducted by limitation of water supply to once per week as follows: 15 ml per pot (mild drought) – D1; 10 ml per pot (moderate drought) – D2, and 5 ml per pot (severe drought) – D3. Control plants were irrigated once per week with optimal volume of water to full soil capacity. After cessation of the stress programme plant's height, fresh weight, and number of green and yellow leaves were recorded.

Biochemical analyses. Samples for biochemical analyses were collected from all leaves which were developed during the drought period (upper than 6th), cut into pieces and immediately frozen in liquid nitrogen. Free proline content was determined according to ninhydrin reagent method of Bates et al. [10]. A

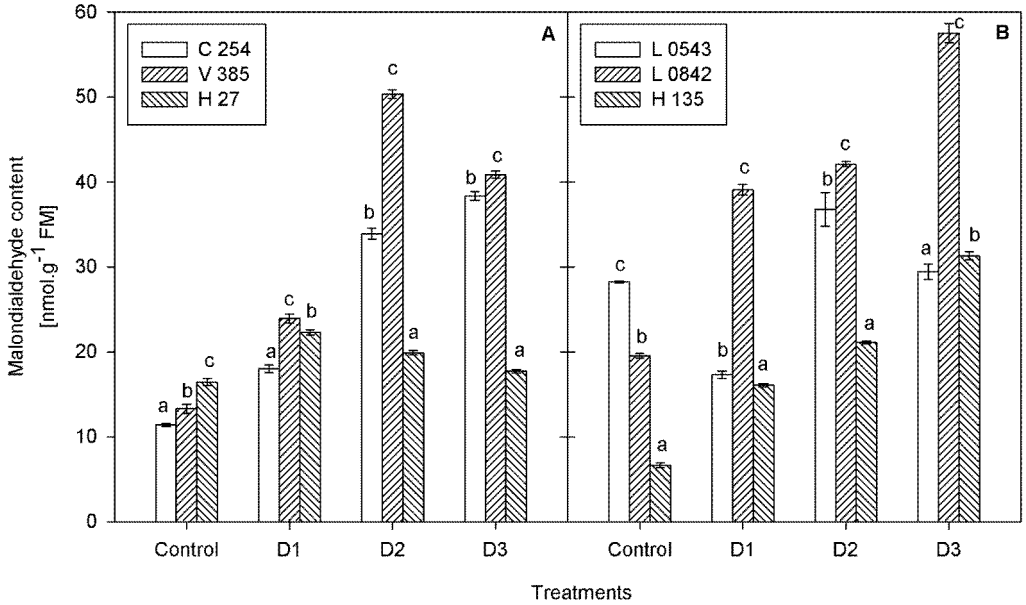

Fig. 1. Malondialdehyde content of Virginia tobacco genotypes Coker 254, Virginia 385, and hybrid H 27 (A), line L 0543, line L 0842, and hybrid H 135 (B) subjected to mild (D1), moderate (D2), and severe (D3) drought stress for 4 weeks. Data presented are mean values ± standard error. Different small letters designate statistically significant differences between genotypes in each treatment group

thiobarbituric acid test [11] was used to assess lipid peroxidation in tobacco plant leaves, measuring content of malondialdehyde (MDA) as the endpoint product. Content of hydrogen peroxide was measured according to the method of ALEXIEVA et al. [12].

Statistics. The experiment was repeated three times. All data are presented as mean value ± standard error (SE). The data were analyzed by one-way ANOVA with post-hoc Duncan's multiple range test at $p < 0.05$.

Results. Biochemical assessment of the drought tolerance of different tobacco genotypes was done accounting the alterations in the content of MDA – a typical stress biomarker (Fig. 1). Both introduced genotypes Coker 254 and Virginia 385 gradually increased MDA content depending on stress severity as compared to control plants (Fig. 1A). Similar trend of alteration of MDA was found in Bulgarian lines (Fig. 1B), especially in L 0842, in which MDA content reached highest value (more than twice as compared to control) after severe drought (D3). However, in both hybrids H 27 and H 135 the MDA content was lower than that of parental genotypes, showing less impairment of cell bio-membranes due to the stress applied.

The increase in the amount of hydrogen peroxide is another reliable indicator of the development of free radical processes in cells, i.e. oxidative stress. Significant

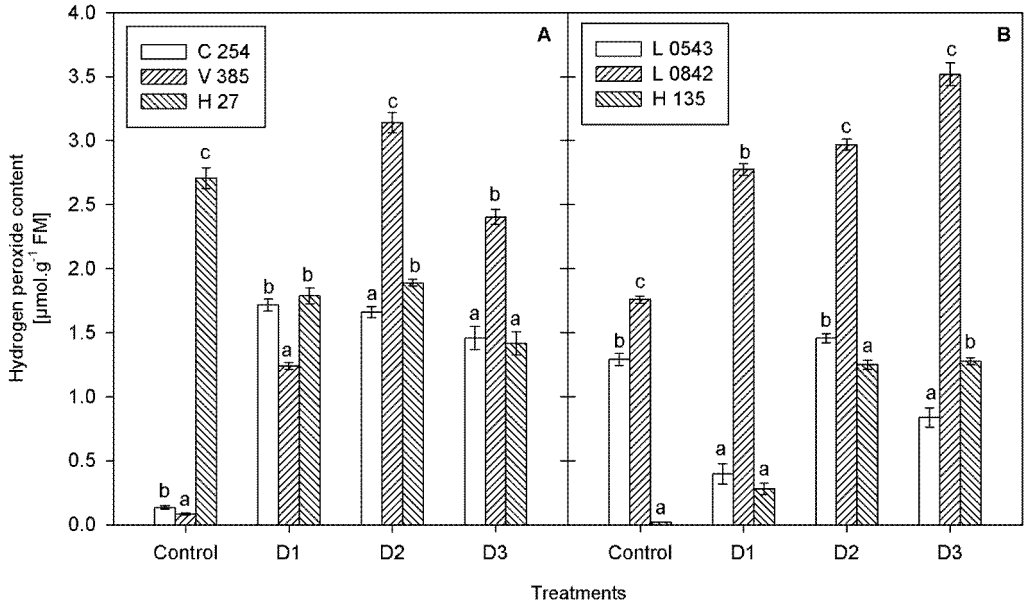

Fig. 2. Hydrogen peroxide content of Virginia tobacco genotypes Coker 254, Virginia 385, and hybrid H 27 (A), line L 0543, line L 0842, and hybrid H 135 (B) subjected to mild (D1), moderate (D2), and severe (D3) drought stress for 4 weeks. Data presented are mean values ± standard error. Different small letters designate statistically significant differences between genotypes in each treatment group

accumulation of hydrogen peroxide due to drought stress was detected in both introduced tobacco cultivars (Fig. 2A) as well as in the Bulgarian line L 0842 (Fig. 2B), while in line L 0543 the content of hydrogen peroxide was decreased or not significantly changed. Different tendencies of H_2O_2 alteration were observed in the hybrids – in H 27 hydrogen peroxide was decreased after drought, while in H 135 it was noticeably increased as compared to the corresponding control amounts.

Free proline is a cell osmolyte, which accumulates under stress conditions. Significant accumulation of proline amount due to drought stress was detected in the introduced tobacco genotypes Coker 254 and Virginia 385 – up to 6-fold after severe drought, and in lesser degree in their hybrid H 27 (Fig. 3A). Similar trends were observed in the Bulgarian tobacco lines (especially in L 0842), and the corresponding hybrid H 135 (Fig. 3B).

The number of green leaves developed during the stress period in all tobacco genotypes and their corresponding hybrids decreased as compared to the control plants (Table 1). This was accompanied by an increase of the number of yellow leaves except for cultivar Coker 254 and line L 0543. Both hybrid progenies H 27 and H 135 had similar morphometric alterations as those of their parental genotypes Coker 254 and line L 0543, unlike the other two parental genotypes

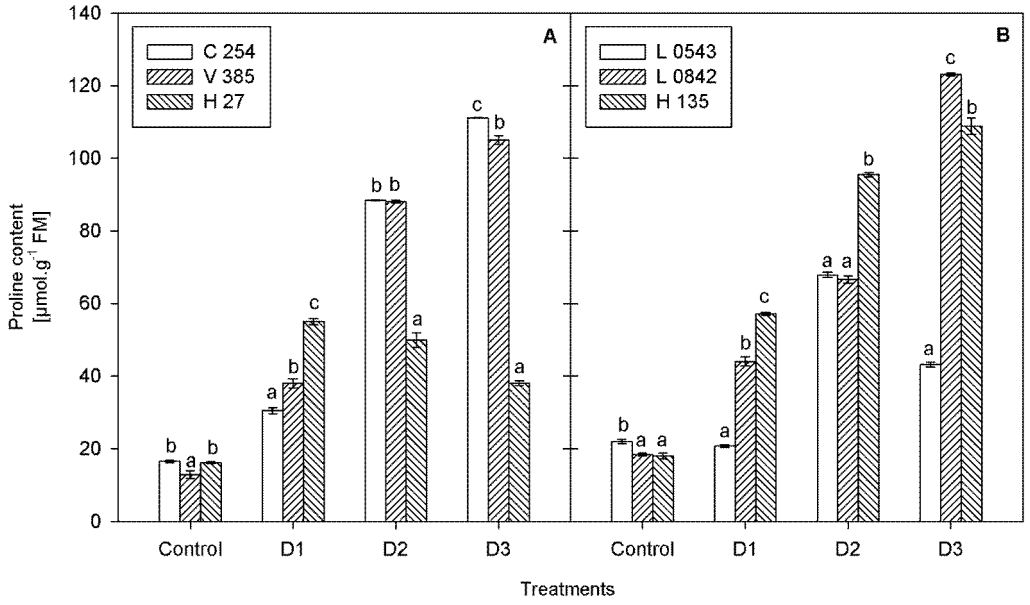

Fig. 3. Free proline content of Virginia tobacco genotypes Coker 254, Virginia 385, and hybrid H 27 (A), line L 0543, line L 0842, and hybrid H 135 (B) subjected to mild (D1), moderate (D2), and severe (D3) drought stress for 4 weeks. Data presented are mean values ± standard error. Different small letters designate statistically significant differences between genotypes in each treatment group

Virginia 385 and L 0842. The tendencies of changes in the number of yellow and green leaves due to water stress deepened with the severity of drought applied. Drought stress retarded also plant's growth and accumulation of fresh biomass of all tobacco genotypes (Table 1). This negative impact intensified also with strengthening of the stress.

Discussion. Drought affected in different way Virginia tobacco genotypes in terms of the parameters studied. Malondialdehyde (MDA) is a cytotoxic product of lipid peroxidation and is an indicator of the cell damage due to the production of free radicals. It is a widely used marker the concentration of which varies in response to biotic and abiotic stresses [13]. Susceptibility to water stress in *Avena* species has been reported on the base of high MDA content [14]. Cvikrová et al. [15] observed a slight increase in MDA levels in the upper leaves of wild tobacco during the stress period and suggested that active defense mechanisms render better protection against oxidative damage in these tissues. We found that MDA content increased in all tobacco genotypes due to drought, but the increment was smallest in both newly-bred hybrids as compared to their corresponding parental genotypes. This showed that stress provoked less damage in hybrid progenies and probably they are more tolerant to drought as compared to the other varieties.

Hydrogen peroxide is generated in plant cells during the normal physiological processes and each deviation in its levels is an early, general response of plants to

Table 1

Morphometric traits of tobacco genotypes subjected to drought for 4 weeks

Genotype/ treatment		Number of leaves developed during the stress period		Plant height (mm)	Plant fresh weight (g)
		Yellow	Green		
C 254	C	0.0 ± 0.00^{a}	5.8 ± 0.20^{ab}	52.4 ± 4.08^{b}	17.10 ± 1.20^{a}
V 385	C	0.2 ± 0.20^{a}	5.4 ± 0.24^{a}	35.2 ± 3.47^{a}	16.05 ± 0.65^{a}
H 27	C	0.0 ± 0.00^{a}	6.0 ± 0.00^{b}	67.0 ± 2.07^{c}	26.15 ± 1.52^{b}
C 254	D1	0.0 ± 0.00^{a}	3.0 ± 0.32^{a}	27.6 ± 1.21^{c}	5.41 ± 0.77^{b}
V 385	D1	0.8 ± 0.20^{b}	3.0 ± 0.32^{a}	21.8 ± 1.53^{b}	4.61 ± 0.29^{b}
H 27	D1	0.0 ± 0.00^{a}	2.4 ± 0.40^{a}	18.4 ± 0.93^{a}	3.28 ± 0.38^{a}
C 254	D2	0.0 ± 0.00^{a}	2.4 ± 0.40^{b}	18.8 ± 1.46^{b}	2.25 ± 0.14^{c}
V 385	D2	1.8 ± 0.20^{c}	1.6 ± 0.24^{a}	16.4 ± 0.40^{a}	1.60 ± 0.09^{b}
H 27	D2	1.0 ± 0.32^{b}	1.25 ± 0.25^{a}	14.6 ± 1.63^{a}	1.10 ± 0.22^{a}
C 254	D3	0.0 ± 0.00^{a}	2.2 ± 0.20^{b}	17.4 ± 0.51^{ab}	1.87 ± 0.23^{a}
V 385	D3	1.6 ± 0.24^{b}	1.6 ± 0.24^{a}	19.4 ± 0.81^{b}	2.56 ± 0.30^{a}
H 27	D3	0.2 ± 0.20^{a}	2.4 ± 0.40^{b}	15.0 ± 1.95^{a}	1.96 ± 0.43^{a}
L 0543	C	0.0 ± 0.00^{a}	6.4 ± 0.24^{b}	76.0 ± 3.73^{c}	17.51 ± 1.00^{a}
L 0842	C	0.0 ± 0.00^{a}	5.6 ± 0.24^{a}	46.0 ± 4.44^{a}	18.94 ± 2.44^{a}
H 135	C	0.0 ± 0.00^{a}	5.8 ± 0.20^{a}	54.4 ± 3.12^{b}	16.39 ± 0.48^{a}
L 0543	D1	0.0 ± 0.00^{a}	3.4 ± 0.24^{c}	36.0 ± 1.45^{b}	3.09 ± 0.32^{b}
L 0842	D1	0.2 ± 0.20^{a}	1.4 ± 0.24^{a}	18.4 ± 0.81^{a}	2.13 ± 0.35^{a}
H 135	D1	0.0 ± 0.00^{a}	2.8 ± 0.20^{b}	19.8 ± 0.66^{a}	2.40 ± 0.12^{a}
L 0543	D2	0.0 ± 0.00^{a}	1.8 ± 0.37^{a}	29.0 ± 2.85^{b}	1.84 ± 0.42^{b}
L 0842	D2	0.4 ± 0.24^{b}	2.0 ± 0.00^{a}	16.6 ± 1.03^{a}	1.84 ± 0.50^{b}
H 135	D2	0.0 ± 0.00^{a}	2.2 ± 0.20^{a}	15.6 ± 1.17^{a}	1.31 ± 0.12^{a}
L 0543	D3	0.0 ± 0.00^{a}	2.4 ± 0.68^{b}	27.4 ± 6.87^{c}	2.45 ± 0.57^{c}
L 0842	D3	1.4 ± 0.24^{b}	0.4 ± 0.24^{a}	13.4 ± 1.21^{a}	0.93 ± 0.31^{a}
H 135	D3	0.2 ± 0.20^{a}	2.0 ± 0.32^{b}	18.8 ± 1.83^{b}	1.47 ± 0.16^{b}

Legend: C – control; D1 – mild drought stress; D2 – moderate drought stress; D3 – severe drought stress. All data presented are mean values ± standard error. Different small letters designate statistically significant differences between genotypes in each treatment group

the occurrence of various stressors. Different types of stress lead to rapid synthesis of hydrogen peroxide in cell organelles, and its increased levels are often used as a biochemical marker for the presence of adverse oxidative stress. However, hydrogen peroxide plays a dual role in plants, at high concentrations it is harmful to the cells, but at low concentrations, it acts as a signalling molecule, which triggers defense mechanisms leading to tolerance against biotic and abiotic stresses [16]. Hydrogen peroxide rose due to drought in the introduced parental genotypes and in the Bulgarian line L 0842, which showed occurrence of oxidative events in these tobaccos. On the contrary, smaller increase of H_2O_2 in H 135, no change in L 0543, and even decrease in H 27 suggested that less oxidative stress occurred in these tobacco plants. Possible participation of H_2O_2 in signalling pathways boosting antioxidant defense could not be excluded in these genotypes.

The lower contents of H_2O_2 and MDA in the hybrid genotypes, as well as in the parental genotypes Coker 254 and L 0543 correlated well with the absence or low number of yellow leaves, and less decrease in plant height and fresh weight under all stress conditions (Table 1). On this basis we could suggest that these Virginia tobacco genotypes are more tolerant to drought stress. The other two tobacco genotypes Virginia 385 and L 0842 had worsened morphometric traits (smallest number of green leaves, biggest number of yellow leaves, and substantial decrease of fresh weight and plant height of L 0842), which were probably linked to their higher amount of MDA and H_2O_2. These findings indicate that these tobacco genotypes are more susceptible to drought.

Proline is an amino acid that accumulates in plants in response to a wide range of biotic and abiotic stress [17]. Proline acts as an osmolyte, scavenges hydroxyl radicals, increases oxygen and provides energy for growth, thus protects cells from damage and helps the plant to relieve stress [13]. Its accumulation in plant cells has been proposed as a general indicator of tolerance to water deficiency and used in the selection of tolerant plant varieties to osmotic stress [8, 15]. Masheva [18] studied the differences in the levels of proline concentration during drought and recovery of seven varieties of oriental tobacco and their hybrids and found that the initial proline levels in tolerant varieties were lower than those in drought-sensitive varieties. Under conditions of water stress, most of the tolerant cultivars accumulated significantly higher proline amount, which allowed them to compensate for the unfavourable water deficit. Similar data were reported by Shtereva et al. [19] for an increase in proline content in L 0842 under drought stress. Although we also found that this genotype accumulated highest amount of proline under drought conditions, it also had highest content of other stress markers (MDA and H_2O_2), and worsened phenotypic characteristics. On the basis of the results obtained we considered that this Bulgarian line L 0842 is moderately susceptible to drought. Similar suggestion can be done for Virginia 385. Coker 254, L 0543, and H 135 tobacco genotypes also accumulated high amounts of proline, while in H 27 hybrid the increase was the lowest. However, this hybrid had the smallest content of stress markers. Probably other components of antioxidant defense (both enzymatic and non-enzymatic) were triggered to compensate the smaller increase of proline and to ensure drought resistance.

Drought stress induces growth retardation and various physiological responses in plants. The reactions of plants to water stress differ significantly depending on the intensity of stress, as well as on the plant genotypes. Celik and Atak [20] reported that the drought response of tobacco was strongly influenced by genetic factors. Our results are similar to those findings and we can conclude that both hybrids are more tolerant to drought stress than their parental genotypes, and especially than Virginia 385 and L 0842, which can be classified as moderately susceptible to drought. The lower level of MDA and H_2O_2 in the newly-developed hybrids H 27 and H 135 as compared to one or both parental genotypes indicated

that the hybrids have better protection against the drought-induced oxidative damages. This was supported also by good morphometric traits measured in the hybrid plants grown under drought stress conditions as compared to the respective father parental genotypes Virginia 385 and L 0842. Further in-depth analysis of the protective mechanisms to drought of hybrid Virginia tobaccos is important and will serve in future breeding programmes for selection of tolerant genotypes.

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