

Biochemical assessment of the tolerance to soil drought of Virginia tobacco varieties

Marina Drumeva-Yoncheva¹, Elena Shopova², Bistra Mihailova², Dessislava Todorova^{2*}, Iskren Sergiev² and Yonko Yonchev³

¹ Agricultural Academy, Tobacco and Tobacco Products Institute, 4108 Markovo, Bulgaria

² Bulgarian Academy of Sciences, Institute of Plant Physiology and Genetics, 1113 Sofia, Bulgaria

³ Agricultural Academy, Fruit Growing Institute, 4004 Plovdiv, Bulgaria

*Corresponding author: dessita@bio21.bas.bg

Abstract

Drumeva-Yoncheva, M., Shopova, E., Mihailova, B., Todorova, D., Sergiev, I. & Yonchev, Yo. (2024). Biochemical assessment of the tolerance to soil drought of Virginia tobacco varieties. *Bulg. J. Agri. Sci.*, 30(1), 37–42

Soil drought is among the serious problems in agriculture leading to considerable losses of crop production. Tobacco is important industrial crop, which is relatively sensitive to water deficit, and the selection of drought resistant varieties is of particular interest. In line with this, we examined the drought tolerance of three genotypes of flue-cured Virginia tobacco – cv. Coker 254 (American origin), line L 825 (Czech origin), which are introduced in Bulgaria, and their hybrid cv. Virzhinia 0514, which is certified as Bulgarian standard. The content of stress biomarkers malondialdehyde, hydrogen peroxide and free proline as well as some morphometric traits were assessed in plants subjected to three levels of drought. The results showed that the three tested tobacco genotypes were moderately tolerant to drought. These data can be used for selection and development of new genotypes with improved tolerance to soil drought.

Keywords: *Nicotiana tabacum* L.; hydrogen peroxide; malondialdehyde; proline; water deficiency

Introduction

Unfavorable environmental conditions can cause considerable losses of agricultural production worldwide. Drought is one of the major problems in the contemporary agriculture, as this abiotic stress impedes crops from realizing their genetic potential (Zhu, 2002). It provokes various physiological reactions in plants at distinct level – from molecular base to whole organism. Drought-induced plant responses vary significantly depending on the crop species, the plant development stage, and the stress duration and severity (Chaves et al., 2003).

Broad-leaved tobacco (*Nicotiana tabacum* L.) is one of the important industrial crops in the world. Originating from the subtropics, it is vulnerable to drought during the early leaf development and growth (Su et al., 2017). Insuf-

ficient water supply disrupts key physiological processes and retards tobacco growth, reduces plant height and leaf area (Biglouei et al., 2010). It was found that during the periods of rapid growth of leaves and stems (during the sixth and seventh week after transplanting the plants) drought is most harmful to the growth of tobacco (Maw et al., 2009).

The lack of steady rainfall and appropriate irrigation under field conditions can be overcome by breeding of varieties that tolerate drought (Korubin-Aleksoska, 2017). Drought tolerant genotypes sustain high productivity and yield even when grown under conditions of severe drought (Blum, 2005). Investigation of plant response mechanisms linked to drought resistance is an important part of the development of stress-tolerant crops (Reddy et al., 2004). A rapid and easy laboratory screening method for testing stress tolerance is the assessment of the content of distinctive stress-related bi-

omarkers like malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and proline (Shtereva et al., 2017). Usually, under stress conditions, their level of accumulation is dramatically elevated, which is a reliable indicator of plant stress (Todorova et al., 2021).

Large-leaved Virginia tobacco is cultivated in Bulgaria mainly in irrigated regions. Most of flue-cured cultivars grown in Bulgaria are introduced but some are created in the Tobacco and Tobacco Products Institute (TTPI), Markovo, Bulgaria by heterosis hybridization and breeding schemes.

This study aimed to compare the tolerance to drought of Virginia tobacco genotypes with different geographic origin (American and Czech) and their hybrid – officially registered Bulgarian flue-cured tobacco variety by measuring stress biomarkers content and some morphogenic traits.

Materials and Methods

Plant material and treatments

We studied the drought tolerance of flue-cured Virginia tobacco (*Nicotiana tabacum* L.) plants – cv. Coker 254 (American origin), line L825 (Czech origin), and their hybrid progeny Bulgarian cv. Virzhinia 0514 (Chinchev et al., 1991), which is official variety used as a tobacco standard in Bulgaria. Seeds of all three genotypes were initially germinated in a Float System for Producing Tobacco Seedlings. Then the seedlings were grown in a growth chamber as a soil culture under following controlled conditions – 16/8h photoperiod, light density $150 \mu\text{mol m}^{-2}\text{s}^{-1}$, 75% relative air humidity and temperature $25^\circ/18^\circ\text{C}$ day/night. Plants were transplanted to individual pots (65/65/60 mm) at the 4th true leaf stage and grown under the same growth conditions. Five uniform plants at the 6th true leaf stage of each genotype were subjected to three level of water deficit treatment with duration of 4 weeks. Water supply was to once per week according to the following schedule: D1 mild drought (15 ml per pot); D2 moderate drought (10 ml per pot); D3 severe drought (5 ml per pot). The control plants were irrigated also once per week but with water sufficient to reach the full water retaining soil capacity. Phenotype traits like plant fresh weight, plant height and number of green and yellow leaves were recorded after cessation of the drought stress.

Biochemical analyses

From the leaves, which developed during the drought stress period samples for analyses were collected. The fresh leaf material was cut, weighed and frozen in liquid nitrogen. The content of malondialdehyde (MDA) is a reliable parameter reflecting the biomembrane integrity. Its concentration was measured according to the method of Kramer et

al. (1991). The levels of hydrogen peroxide were measured according to the experimental procedure of Alexieva et al. (2001). The free proline content was determined after acid derivatization with ninhydrin reagent following the procedure described by Bates et al. (1973).

Statistics

The data presented are mean values \pm standard error. The experiments were repeated three times in three replicates each. The significance of the differences between treatments were assessed by one-way ANOVA with Duncan's multiple range post-hoc analysis at $p < 0.05$.

Results

Malondialdehyde content was altered in a distinct way due to the drought stress in all three tobacco genotypes studied (Figure 1). A substantial increase in the MDA level was detected in the American cultivar Coker 254 and it was nearly 3.5 times higher in D3-stressed plants than the control. MDA did not alter significantly, or decreased after drought treatment in the Czech line L 0825. In the Bulgarian variety V 0514, MDA also gradually increased in term of drought severity and reached nearly 2 times higher values after severe drought (D3) as compared to the control.

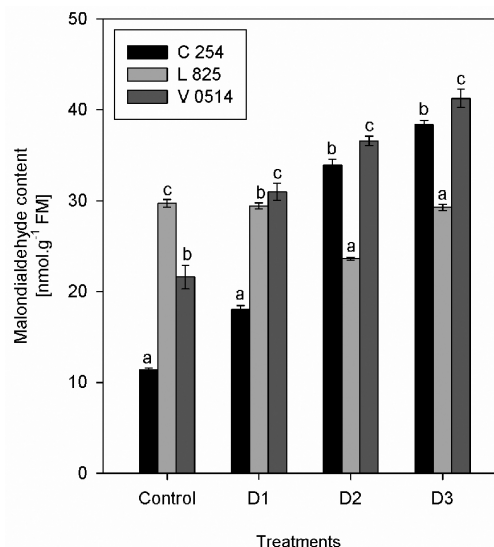


Fig. 1. Malondialdehyde content of Virginia tobacco genotypes Coker 254 (American), line L 825 (Czech), and Virzhinia 0514 (Bulgarian), subjected to mild (D1), moderate (D2), and severe (D3) drought stress for 4 weeks. Data presented are mean values \pm standard error. Different small letters designate statistically significant differences between genotypes in each treatment group

Hydrogen peroxide content was increased in all three genotypes due to the drought stress applied (Figure 2). In the American tobacco Coker 254 the increase of H_2O_2 was about 12.7 to 10.7-fold higher than the control due to mild (D1) or severe drought (D3). Mild drought did not change hydrogen peroxide content in the Czech genotype L 825, while it was increased by 2.5-fold after severe drought. In the Bulgarian variety V 0514, the augmentation of H_2O_2 was smallest and in D3-treated plants it was only 43% higher than the control. It should be noted that V 0514 showed a characteristic high background level of hydrogen peroxide in the control plants.

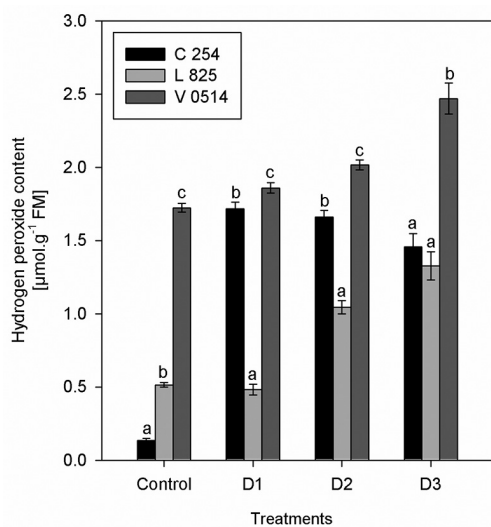


Fig. 2. Hydrogen peroxide content of Virginia tobacco genotypes Coker 254 (American), line L 825 (Czech), and Virzhinia 0514 (Bulgarian), subjected to mild (D1), moderate (D2), and severe (D3) drought stress for 4 weeks. Data presented are mean values \pm standard error. Different small letters designate statistically significant differences between genotypes in each treatment group

Free proline content was not changed considerably after drought stress treatment in the Czech L 825 tobacco genotype (Figure 3). In the American cultivar Coker 254 it gradually increased depending on drought severity, and after severe drought it reached value of 6.7-fold higher as compared to the control. In the Bulgarian genotype V 0514, the free proline content was increased by 3.3 – 3.6-fold due to the drought.

The growth of the studied tobacco cultivars was considerably retarded due to the drought stress applied (Figure 4). The negative impact of drought intensified with increase of its severity. Most obvious decrease was detected in the Czech line L 825 in which the plant height was reduced

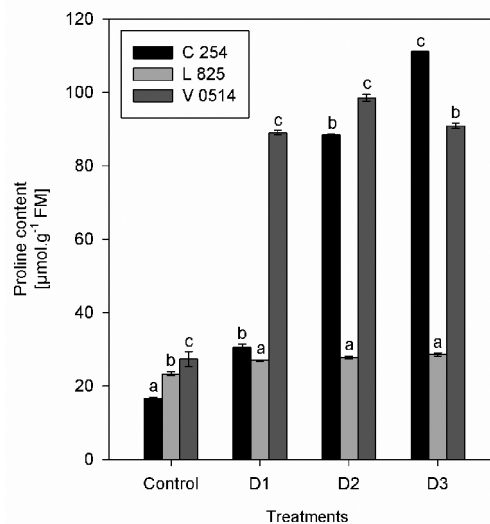


Fig. 3. Free proline content of Virginia tobacco genotypes Coker 254 (American), line L 825 (Czech), and Virzhinia 0514 (Bulgarian), subjected to mild (D1), moderate (D2), and severe (D3) drought stress for 4 weeks. Data presented are mean values \pm standard error. Different small letters designate statistically significant differences between genotypes in each treatment group

nearly 5 times and fresh weight of the plants – more than 16 times after severe drought (D3) as compared to the control. The decrease of plant fresh weight and height detected in Coker 254 and Virzhinia 0514 was comparable.

It was established that the number of newly developed green leaves substantially decreased in the plants of all three tobacco genotypes subjected to drought as compared to the normally irrigated plants (Figure 5). No yellow leaves were developed during the stress period in the three tobacco genotypes (data not shown).

Discussion

Similarly to other abiotic environmental stress factors, drought causes oxidative stress due to increased generation of reactive oxygen species (ROS) – superoxide ($O_2^{\cdot-}$) and the hydroxyl radicals ($\cdot OH$), hydrogen peroxide (H_2O_2) and singlet oxygen (1O_2) (Gill & Tuteja, 2010; Hasanuzzaman et al., 2020). These ROS can alter the normal cellular metabolism and can induce oxidative damage to cell biomembranes (Gill & Tutjea, 2010; Hasanuzzaman et al., 2020). Malondialdehyde, an end product of the lipid peroxidation of the unsaturated fatty acids, has been considered as bio-indicator of oxidative damage and its accumulation has been used to distinguish drought-sensitive and drought-

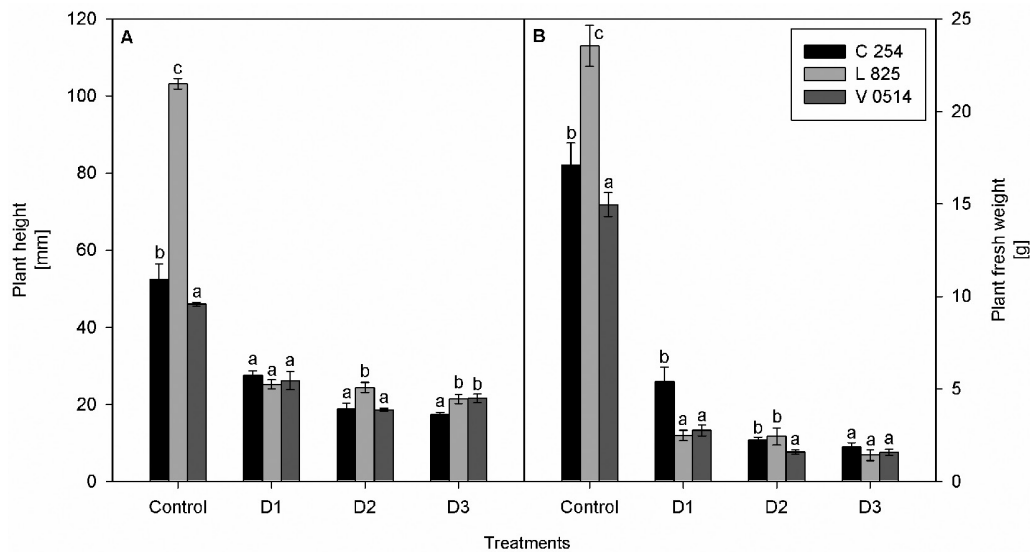


Fig. 4. Plant height and fresh weight of Virginia tobacco genotypes Coker 254 (American), line L 825 (Czech), and Virzhinia 0514 (Bulgarian), subjected to mild (D1), moderate (D2), and severe (D3) drought stress for 4 weeks. Data presented are mean values \pm standard error. Different small letters designate statistically significant differences between genotypes in each treatment group

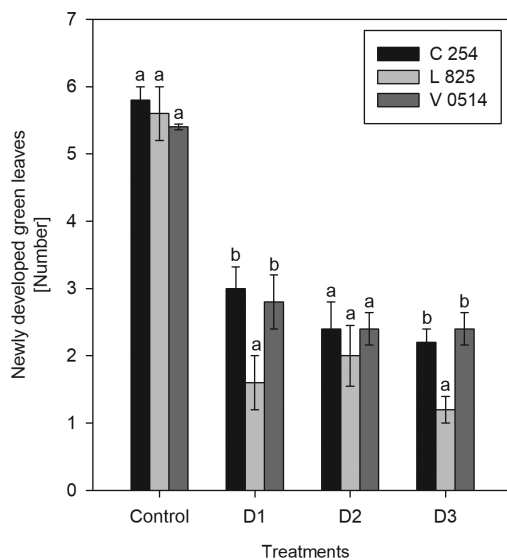


Fig. 5. Number of newly developed green leaves (after 6th) in Virginia tobacco genotypes Coker 254 (American), line L 825 (Czech), and Virzhinia 0514 (Bulgarian), subjected to mild (D1), moderate (D2), and severe (D3) drought stress for 4 weeks. Data presented are mean values \pm standard error. Different small letters designate statistically significant differences between genotypes in each treatment group

tolerant plant varieties (Sairam & Srivastava, 2001; Liu et al., 2014). Likewise, H_2O_2 accumulation has been reported in response to drought and has been also utilized as stress-related indicator (Sairam & Srivastava, 2001; Apel & Hirt, 2004). *In planta* hydrogen peroxide plays a dual role. It is

harmful to the cells at high concentrations, while in low concentrations it acts as a signalling molecule and triggers the antioxidant system to cope with stress (Foyer & Noctor, 2005). We found that the American genotype Coker 254 and the Bulgarian variety Virzhinia 0514 possessed higher amounts of MDA under drought conditions as compared to the normally irrigated controls (Figure 1) suggesting some biomembrane deterioration in these cultivars. Interestingly, MDA content did not change in the Czech line L 825 during all treatments, indicating greater cell membrane integrity and less membrane injuries. Due to water deprivation hydrogen peroxide was greatly increased in Coker 254 and L 825, and in less degree in Virzhinia 0514 (Figure 2). This probably indicates that oxidative stress was generated because of enormous ROS production caused by water deficiency but it was differentially expressed in the different tobacco genotypes. Our results are in line with the findings of other authors, where MDA and H_2O_2 content in tobacco and wheat increased gradually with the severity of the water deficit (Cvikrova et al., 2013; Shtereva et al., 2017; Todorova et al., 2021; Shopova et al., 2022). However, the phenotypic alterations of tobacco genotypes Coker 254 and Virzhinia 0514 showed less decrease in the number of newly developed leaves (Figure 5), plant fresh weight and height (Figure 4) under all stress conditions as compared to those of the Czech line 825. These findings suggested that although the membrane injuries were significant in the American and Bulgarian tobacco genotypes, which was witnessed by higher MDA content, plants had a compensatory mechanism ensuring further plant survival and growth.

During drought, plants withstand to oxidative stress through accumulation of various osmolytes like glycine be-

taine, proline, sugars, etc. (Basu et al., 2016). Although proline is discussed as stress biomarker, this predominant solute also plays role in protection of plants from water deficit. The capability of plants to accumulate proline under adverse environment has been shown to be in line with their resistance to stress (Zaifnejad et al. 1997; Dobra et al., 2011; Liu et al., 2014). Proline content in the American Coker 254 and Bulgarian Virzhinia 0514 tobacco genotypes was greatly increased due to the water stress (Figure 3). Most probably, the higher levels of proline in these cultivars helped to prevent them from injury and maintain better growth under drought stress. On the opposite, no significant increase of proline was detected in the Czech line 825 (Figure 3), along with minor membrane injuries (Figure 1) and increased level of hydrogen peroxide (Figure 2). These results suggest that Czech line 825 probably possessed a powerful ROS scavenging defense system consisted of diverse antioxidants (both enzymatic and non-enzymatic), which was triggered by H_2O_2 to prevent membrane deteriorations. Additional analyses on plant defense mechanisms are required to confirm our observation.

Conclusions

Based on the results obtained it could be summarized that all three tobacco genotypes showed relatively good tolerance to drought stress. Although relatively high levels of oxidative stress (MDA and hydrogen peroxide) were detected in the American variety Coker 254, this genotype showed the best growth characteristics under stress conditions, which correlated with the increased levels of free proline. The growth parameters of the Czech line were most affected, but insignificant change and even decrease in the level of MDA due to drought was observed. As a hybrid of the two introduced genotypes, the Bulgarian variety combines their positive characteristics, manifesting as less increase in stress markers MDA and hydrogen peroxide, similar to the Czech parent, and a relatively high level of proline and growth parameters comparable to those of the American parent under conditions of water deficit. These results correlate with the observations of Celik & Atak (2012), who reported that the response of tobacco to stress could be genetically determined.

Our results demonstrate the advantages of Virzhinia 0514 over the introduced tobacco genotypes and confirm the proper selection of parental components with good adaptability to the climate conditions of Bulgaria. The results of the study could be of interest for preliminary screening of drought-tolerant flue-cured Virginia tobacco genotypes capable to withstand moderate water deprivation.

References

- Alexieva, V., Sergiev, I., Mapelli, S. & Karanov, E. (2001). The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell Environ.*, 24, 1337–1344.
- Apel, K. & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Ann. Rev. Plant Biol.*, 55, 373–399.
- Basu, S., Ramegowda, V., Kumar, A. & Pereira, A. (2016). Plant adaptation to drought stress. *F1000Research*, 5, 1554.
- Bates, L. S., Waldren, R. P. & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant Soil*, 39, 205–207.
- Biglouei, M. H., Assimi, M. H. & Akbarzadeh, A. (2010). Effect of water stress at different growth stages on quantity and quality traits of Virginia (flue-cured) tobacco type, *Plant Soil Environ.*, 56(2), 67–75.
- Blum, A. (2005). Drought resistance, water – use efficiency and yield potential — are they compatible, dissonant or mutually exclusive. *Austr. J. Agricult. Res.*, 56, 1159–1168.
- Celik, O. & Atak, C. (2012). The effect of salt stress on antioxidative enzymes and proline content of two Turkish tobacco varieties. *Turk. J. Biol.*, 36, 339–356.
- Chaves, M. M., Maroco, J. P. & Pereira, J. S. (2003). Understanding plant responses to drought – from genes to the whole plant. *Funct. Plant Biol.*, 30(3), 239–264.
- Chinchev, B., Stoyanov, B. & Ralovski, S. (1991). Agrobiological, technological and chemical characteristics of Virginia tobacco varieties. *Bulgarian Tobacco*, 1, 2–6 (Bg).
- Cvikrova, M., Gemperlovaá, L., Martincova, O. & Vankova, R. (2013). Effect of drought and combined drought and heat stress on polyamine metabolism in proline-over-producing tobacco plants. *Plant Physiol. Biochem.*, 73, 7–15.
- Dobra, J., Vankova, R., Havlova, M., Burman, A. J., Libus, T. & Storchova, H. (2011). Tobacco leaves and roots differ in the expression of proline metabolism-related genes in the course of drought stress and subsequent recovery. *J. Plant Physiol.*, 168, 1588–1597.
- Foyer, C. H. & Noctor, G. (2005). Oxidant and antioxidant signaling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant Cell Environ.*, 29, 1056–1071.
- Gill, S. S. & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.*, 48, 909–930.
- Hasanuzzaman, M., Bhuyan, M. H. M. B., Parvin, K., Bhuiyan, T. F., Anee, T. I., Nahar, K., Hossen, S., Zulfiqar, F., Alam, M. & Fujita, M. (2020). Regulation of ROS metabolism in plants under environmental stress: A review of recent experimental evidence. *Int. J. Mol. Sci.*, 21, 8695.
- Korubin-Aleksoska, A. (2017). Breeding drought tolerance in tobacco. *Tobacco*, 67(1-6), 13–22.
- Kramer, G. F., Norman, H. A., Krizek, D. T. & Mirecki, R. M. (1991). Influence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids in cucumber. *Phytochemistry*, 30, 2101–2108.
- Liu, J., Li, J., Su, X. & Xia, Z. (2014). Grafting improves drought tolerance by regulating antioxidant enzyme activities and

- stress-responsive gene expression in tobacco. *Environ. Exp. Bot.*, 107, 173–179.
- Maw, B. W., Stansell, J. R. & Mullinix, B. G.** (2009). Soil-plant-water relationships for flue-cured tobacco. University of Georgia. *Res. Bull. Georgia Agricult. Exp. Station*, 427, 40.
- Reddy, A. R., Chaitanya, K. V. & Vivekanandan, M.** (2004). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol.*, 161, 1189–1202.
- Sairam, R. K. & Srivastava, G. C.** (2001). Water stress tolerance of wheat (*Triticum aestivum* L.): variations in hydrogen peroxide accumulation and antioxidant activity in tolerant and susceptible genotypes. *J. Agron. Crop Sci.*, 186(1), 63-70.
- Shopova, E., Mihailova, B., Drumeva-Yoncheva, M., Todorova, D., Sergiev, I. & Yonchev, Y.** (2022). Comparative study of drought tolerance of Virginia tobacco genotypes differing in origin and their corresponding hybrid progenies. *C. R. Acad. Bulg. Sci.*, 75(10), 1540 -1548.
- Shtereva, L., Stoimenova, E., Drumeva-Yoncheva, M., Michailova, B., Kartzeva, T. & Vassilevska-Ivanova, R.** (2017). Response of Virginia (flue-cured) tobacco genotypes to water-deficit stress. *Arch. Biol. Sci.*, 69(1), 119-127.
- Su, X., Wei, F., Huo, Y. & Xia, Z.** (2017) Comparative physiological and molecular analyses of two contrasting flue-cured tobacco genotypes under progressive drought stress. *Front. Plant Sci.*, 8, 827.
- Todorova, D., Sergiev, I., Katerova, Z., Shopova, E., Dimitrova, L. & Brankova, L.** (2021). Assessment of the biochemical responses of wheat seedlings to soil drought after application of selective herbicide. *Plants*, 10, 733.
- Zaifnejad, M., Clark, R. B. & Sullivan, C. Y.** (1997). Aluminum and water stress effects on growth and proline of sorghum. *J. Plant Physiol.*, 150, 338-344.
- Zhu, J.-K.** (2002). Salt and drought stress signal transduction in plants. *Ann. Rev. Plant Biol.*, 53, 247–273.

Received: November, 29, 2022; Approved: February, 10, 2023; Published: February, 2024