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DROUGHT-INDUCED ACCLIMATION CHANGES IN YOUNG CASTOR (*RICINUS COMMUNIS* L.) PLANTS

Abstract. Castor (*Ricinus communis* L., cv. Donskaya krupnolistnaya) plants were grown as pot-soil culture in a greenhouse of the Agricultural University of Plovdiv, Bulgaria. Young plants (4 leaves stage) were grown for next 15 days at three different soil moisture regimes: optimal - 65-70% field water capacity (FWC) as well as soil moisture deficient regimes, provoking moderate and severe plant water stress. The water regimes were maintained by daily pot weighting and irrigation. The amount of the irrigated water for the plants at optimal water regime was equal to full pot evapotranspiration from the previous day (100% ET), while those for the plants at deficient regimes were equal to 75% ET (moderate water stress) or 50% ET (severe water stress). Both physiological and anatomical parameters were measured at the end of the experiment. It was found the castor plants reacted to drought by development of clearly distinguished xeromorphic traits, which decreased water loss and contributed the optimization of plant water balance at water-deficient conditions. The drought provoked moderate and severe water stress in castor plants, indicated by strong enhancement of leaf GPOD activity. The photosynthetic apparatus of castor plants at drought conditions was preserved. Both photosynthetic pigments content and the apparent electron transport rate (ETR) were slightly diminished and the maximal quantum yield of photosystem II (F_v/F_m) was not affected. The sharp decrease of leaf gas exchange parameters in drought-stressed plants may be considered as a mechanism to avoid possible structural and functional damages.

Key words: castor, drought, growth, leaf lamina, photosynthesis.

Introduction. Castor (*Ricinus communis* L.) is important oilseed crop, cultivated in both tropical and semi-tropical regions of the world (Weiss, 2000). Castor oil is nonedible and has been used almost entirely for industrial and pharmaceutical applications. It is also an important raw material for the chemical and polymer industries, and a promising candidate for biofuel production (Ramos et al., 1984; Ogunniyi, 2006; Mutlu and Meier, 2010). World consumption of castor oil has increased more than 50% during the last 25 years.

As far as castor oil seeds production does not meet the current industrial demand, diversification of castor production regions is necessary. It is known that castor has relatively high level of drought tolerance; therefore its cultivation in drier zones could be a rational option. In this viewpoint, castor has been recently considered as a promising new crop for the South and South-East regions of Republic of Kazakhstan (Назарбаев, 2014).

Plant tolerance to drought usually is based on many different mechanisms. Sowmya et al. (2016) has recently reported that drought tolerant castor genotypes have higher water use efficiency (WUE) due to relatively low transpiration rate. This observation corresponds to the early stomatal closure in the leaves of drought-exposed castor plants and its ability to maintain efficient stomatal control while keeping a high level of net CO₂ fixation (Sausen and Rosa, 2010). The low transpiration rate could be partly due to increased abscisic acid concentration in the phloem sap of this species at drought (Zhong et al., 1996). Babita et al. (2010) reported that osmotic adjustment is another important mechanism for drought tolerance in castor. Nine genotypes of castor subjected to drought demonstrated osmotic adjustments in the leaves, but with a wide variability in the intensity of the effect.

Some plants develop thicker lamina under conditions of water deficiency, allowing them to promote more efficient water use and lower transpiration rate (Lakshamma et al., 2009). There is lack of information for the drought-induced anatomical changes in the leaves of castor plants, and it is unknown what kind of adaptation patterns they develop. This information is important, because there is close relationship between the anatomy adaptations and the efficiency of physiological processes. Therefore, we decided to carry out a study aiming to clarify young castor plants responses and mechanisms of acclimation to drought stress using both physiological and anatomical parameters.

Material and methods. Pot-soil experiments were carried out in the greenhouse of the Department of Plant Physiology and Biochemistry of the Agricultural University of Plovdiv, Bulgaria. Castor cultivar “Donskaya krupnolistnaya” was used in the study. The seeds were sown in pots filled with 4 kg dry soil taken from the experimental field of the University. When plants reached 4 leaf-stage, an experimental design including three variants was set up. Each variant consisted three pots and each pot 4 plants. During the next 15 days plants were grown at different soil moisture regimes – (1) optimal (65-70% field water capacity, FWC) as well as deficient regimes provoking (2) moderate and (3) severe plant water stress. The water regimes were maintained by daily irrigation. The amount of the irrigated water for the plants at optimal water regime was equal to full pot evapotranspiration for previous day (100% ET), while those for plants at deficient regimes were equal to 75% ET (moderate water stress) and 50% ET (severe water stress). At the end of the experiment, following measurements of growth, physiological, and anatomical parameters were made.

Plant growth determination. Fresh weights of the plants as well as their leaf area were determined at the end of experiment. The leaf area was measured by an electronic area meter (NEO-2, TU-Sofia, Bulgaria).

Guaiacol peroxidase determination. The activity of the antioxidative enzyme guaiacol peroxidase (GPOD) in the leaves was measured by methods of Bergmeyer (1974).

Anatomical analysis. Samples from roots and leaves were collected at the end of the experiment, fixed in FAA for 24 hours, and transferred for storage in 70% ethyl alcohol. Semi-lasting glycerin slides of epidermal peels and free hand transvers sections were prepared. The observations, measurements, and photographs of the slides were made with a digital light microscope Motic DMBA210 (Motic Incorporation Ltd., China), using the image analysis software Motic Images Plus version 2.0., with a general magnification of X400. The following parameters were examined and analyzed: lamina thickness, palisade and spongy parenchyma height, upper and lower epidermis height, stomatal index (SI), stomata and ordinary epidermal cells (OEC) size, diameter of the root biggest xylem vessel elements. For each examined parameter 30 measurements were made (Hendry et al., 1993).

Leaf gas exchange. Leaf gas exchange (net photosynthetic rate-A, transpiration rate-E, stomatal conductance-gs and internal CO₂ concentration-ci) was measured on the fully developed leaves (closest to the top) at the end of the experimental period with an open photosynthetic system LCpro+ (ADC, England). The measurements were done after one hour adaptation at 550 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 30 °C temperature.

Photosynthetic pigments content. Photosynthetic pigments (chlorophyll a, chlorophyll b and total carotenoids) were extracted in 80 % acetone, measured spectrophotometrically and calculated according to the formulae of Lichtenthaler and Welburn (1983).

Chlorophyll fluorescence. Chlorophyll fluorescence measurements were performed on the fully developed leaves with a pulse modulation fluorometer (MINI-PAM, Heinz Walz, Germany) in dark and light-adapted leaves. The castor plants were kept in the dark for at least 30 min before the start of the measurement. By switching on the measuring beam (0.02 – 0.20 $\mu\text{mol m}^{-2} \text{s}^{-1}$), the minimal level of fluorescence (F_0) was recorded. Immediately thereafter, a saturating light pulse of 5500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with 0.8 s duration was sent out to record the maximal level of fluorescence in the dark-adapted state (F_m), from which the maximal quantum yield of PSII (F_v/F_m) was calculated (with $F_v = F_m - F_0$).

After 30 min light adaptation at 550 $\mu\text{mol m}^{-2} \text{s}^{-1}$ the steady-state level of photosynthesis was achieved and a saturating pulse with the same characteristics was applied. Fluorescence yield before triggering with the saturation pulse (F), maximal (F_m') fluorescence reached during the saturation pulse, effective yield of photochemical energy conversion (Y), $Y = (F_m' - F)/F_m'$ as well as apparent electron transport rate (ETR) calculated as $\text{ETR} = Y * \text{PAR} * 0.5 * 0.84$ (White and Critchley, 1999) were determined.

Statistics. Statistical analysis was performed using a one-way ANOVA (for $P < 0.05$). Based on the ANOVA results, a Tukey test for mean comparison was performed for a 95% confidential level, in order to test for significant differences among treatments. In the tables, different letters (a, b, c) express significant differences. Letter “a” is representing the highest value.

Results and discussion. Castor plants, grown for 15 days at deficient water moisture regimes, grew slowly and formed significantly lower fresh mass (FM) and leaf area (LA) (Table 1). The decrease of FM of plants from the variants 75% ET и 50% ET were 32 and 42.6% and those of LA – 31.9 and 36.9%, respectively. It is important to mention that the diminished values of these parameters in drought-exposed castor plants were mostly due to falling of the lower leaves, which could be considered as a mechanism for water balance optimization.

The results, presented in Table 1, showed sharp increase of GPOD activity in castor plants grown at deficient soil moisture regimes – from over 2 to more than 4-fold. It is well known that GPOD is key enzyme in cell antioxidative network preventing macromolecule oxidation from hydrogen peroxide and other active oxygen species. The enhanced GPOD activity gave evidence that the applied deficient soils moisture regimes provoked moderate (2-fold) and severe (4-fold) water stress in the castor plants.

Table 1 – Effect of different soil moisture regimes on fresh mass, leaf area formation and leaf guaiacol peroxidase activity of the leaves of young *Ricinus communis* plants

Variants	Fresh mass (FM, g/plant)	Leaf area (LA, cm ² plant)	GPOD activity (U/g FM)
100% ET	5.15 ^a	108.2 ^a	4.5 ^c
75% ET	3.51 ^b	74.8 ^b	10.1 ^b
50% ET	2.96 ^b	68.3 ^b	21.8 ^a

Different letters (a, b, c) indicate statistically differences ($P \leq 0.05$), letter “a” representing the highest value.

Plants experiencing drought stress try to acclimate by developing different anatomical and morphological changes and physiological mechanisms. Castor possesses alternate, simple, palmately lobed leaves, with 5–12 deep lobes. The leaf blade is amphistomatous, with uniseriate epidermis, with barrel-shaped ordinary epidermal cells, small anisocytic stomata and higher stomatal frequency on the lower epidermis (Maiti, et al., 2012). The upper epidermis has well developed cuticle, especially in drought-resistant genotypes. Castor plants grown at optimal water regimes have bifacial leaves, with a single layer of palisade parenchyma and 3-5 layers of spongy parenchyma (Figure 1). Cells of the palisade parenchyma are

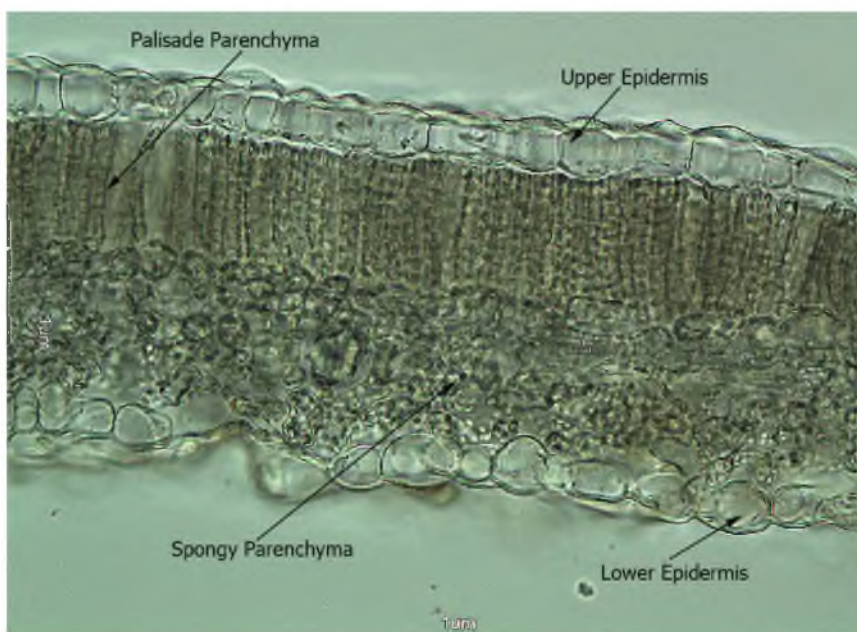


Figure 1 – Bifacial leaf structure of *Ricinus communis* plant, grown at optimal water regime (transverse section, X400)

columnar, thin-walled, with numerous chloroplasts, perpendicularly oriented to the upper epidermis. The cells of the spongy parenchyma are small, thin walled, isodiametric with large intercellular spaces (Tyagi, et al., 2013).

The severe and moderate water stress affected the size of the epidermal cells (Table 2) reducing the height of the upper epidermis with 19% and 8% respectively, and the height of the lower epidermis with 12% and 5% respectively. The water stress did not cause changes in stomata frequency on the upper epidermis, but increased significantly the SI (up to 38%) of the lower epidermis. Increased number of stomata, positioned on the lower leaf surface away from direct sunlight, contributes to a reduction in the rate of loss of absorbed water (Dickison, 2000). A reduction of the size of the stomata and ordinary epidermal cells of the upper and lower epidermis was also observed.

Table 2 – The effect of different soil moisture regimes on *Ricinus communis* leaf anatomical structure

Variants	Leaf lamina				
	Upper epidermis height μ	Lower epidermis height μ	Palisade parenchyma height μ	Spongy parenchyma height μ	Lamina width μ
100% ET	26.29 ^a	22.17 ^a	67.88 ^a	73.44 ^a	191.19 ^a
75% ET	24.22 ^b	21.23 ^a	60.04 ^b	66.51 ^b	171.27 ^b
50% ET	21.30 ^c	19.65 ^b	55.0 ^c	60.96 ^c	159.16 ^c
Variants	Upper epidermis				
	Stomata length μ	Stomata width μ	OEC length μ	OEC width μ	SI %
100% ET	28.01 ^a	19.80 ^a	32.89 ^b	19.23 ^a	11.30
75% ET	28.35 ^a	19.23 ^a	35.95 ^a	19.65 ^a	11.23
50% ET	26.46 ^b	17.55 ^b	34.44 ^b	17.62 ^b	10.03
Variants	Lower epidermis				
	Stomata length μ	Stomata width μ	OEC length μ	OEC width μ	SI %
100% ET	25.74 ^a	19.69 ^a	35.50 ^a	20.85 ^a	14.87 ^b
75% ET	25.66 ^a	18.01 ^b	33.27 ^b	18.96 ^b	20.58 ^a
50% ET	25.35 ^a	17.39 ^b	33.16 ^b	17.83 ^b	19.70 ^a

Different letters (a, b, c) indicate statistically differences ($P \leq 0.05$), letter “a” representing the highest value.

The plants grown under severe and moderate water stress showed statistically significant reduction of the height of the palisade (with 19% and 12% respectively), and spongy mesophyll (with 17% and 10% respectively), which reflected to a reduced size of the lamina (with 17% and 11% respectively). The water stress did not affect the number of the layers composing the palisade and spongy mesophyll but instead reduced the intercellular spaces between the mesophyll cells and made the lamina more compact. It is observed that in some species, a thicker lamina promotes more efficient water use and lower transpiration rates under conditions of water deficiency (Dickison, 2000).

The results showed that castor plants grown under moderate and severe water stress on a histological level reacted to these conditions by developing compact lamina, reduced size of the root xylem vessels (data not shown), ordinary epidermal cells and stomata, palisade and spongy mesophyll, and lamina, and increased stomatal frequency on the lower epidermis - patterns typical for xeromorphic plants. Similar changes in the anatomical structure of the leaf and root of castor plant grown under the influence of industrial effluent were observed by Tyagi et al. (2013). The results of the leaf anatomical studies showed that water stress causes development of clearly distinguished xeromorphic adaptations at a histological level.

Drought stress influenced the performance of castor plants, at physiological level, too. The applied soil moisture deficient regimes reduced significantly leaf gas exchange parameters in drought-stressed plants. The net photosynthetic rate (A) of these plants decreased by 44.1 (moderate stress) and 74.9%

(severe stress), respectively. Transpiration rate showed the same trend, but to a smaller degree. Consequently, water use efficiency (WUE = A/E) in drought-stressed plants also was diminished - from 28 to 25.6%. When stomata limitation prevails over mesophyll constrains, net photosynthetic rate (A) is retarded by low internal CO₂ concentration (ci). In our study, the ci values in drought-exposed castor plants did not vary significantly, which is an indicator for the presence of nonstomatal limitations.

The integral photosynthetic process includes several sub-processes, namely light perception, photosynthetic electron transport, CO₂ assimilation, short and long photoassimilates transport and all of them may be negatively affected by drought. The results, presented in Table 3, showed that the content of photosynthetic pigments was diminished only in plants under severe water stress. The values of both chlorophylls and carotenoids decreased, in average by 25%. The lower degree of drought impact on pigments as compared with net assimilation rate (A) could be partly explained by the fact, that lower and more affected leaves were not used for this analysis as they were fall down.

Table 3 – Effect of different soil moisture regimes on leaf gas exchange, photosynthetic pigment content and selected chlorophyll fluorescence parameters of young *Ricinus communis* plants

Variants	Leaf gas exchange				
	A	E	gs	ci	WUE
100% ET	17.20 ^a	8.12 ^a	0.37 ^a	232 ^a	2.11 ^a
75% ET	9.61 ^b	6.31 ^b	0.21 ^b	254 ^a	1.52 ^b
50% ET	4.32 ^c	2.80 ^c	0.07 ^c	239 ^a	1.57 ^c
Variants	Photosynthetic pigments content and chlorophyll fluorescence parameters				
	Chl.a	Chl.b	Car	F _v /F _m	ETR
100% ET	1.15 ^a	0.43 ^a	0.45 ^a	0.81 ^a	85.1 ^a
75% ET	1.03 ^a	0.37 ^a	0.46 ^a	0.82 ^a	83.1 ^a
50% ET	0.89 ^b	0.32 ^b	0.35 ^b	0.81 ^a	65.2 ^b

A – net photosynthetic rate (μmol CO₂/m²/s); E – transpiration rate (mmol H₂O/m²/s); gs – stomatal conductance (mol/m²/s); ci – internal CO₂ concentration (vpm); WUE – water use efficiency (A/E; μmol CO₂/mmol H₂O); Chl.a,b – chlorophyll content (mg/g FM); Car – carotenoids (mg/g FM); F_v/F_m – maximal quantum yield of photosystem II; ETR – apparent electron transport rate (μmol m⁻² s⁻¹).

Different letters (a, b, c) indicate statistically differences (P ≤ 0.05), letter “a” representing the highest value.

The severe water stress did not influence the maximal quantum yield of photosystem II (F_v/F_m) in castor plants. The measured F_v/F_m values (0.81-0.82) were in the range typical for healthy leaves (0.75 – 0.83). This means that reduced chlorophyll content reflexed the antennae chlorophyll, but not the reaction centers. Nevertheless, the apparent electron transport rate (ETR) was slightly diminished, by 23%. Considering the relatively stable light-dependent photosynthetic processes, as well as the almost equal ci values among the variants, we may conclude that other limiting factors (Calvin cycle biochemical reactions, photosassimilate transport, etc.) may have bigger impact on the photosynthesis of drought-exposed castor plants.

Conclusion. Based on the results obtained we may conclude that young castor plants responded to drought by efficient acclimation at, both, histological and physiological levels. The castor plants reacted to drought by developing clearly distinguished xeromorphic traits, such as compact lamina, reduced size of the root xylem vessels, ordinary epidermal cells and stomata, palisade and spongy mesophyll, and lamina, as well as increased stomatal frequency on the lower epidermis. All these changes decreased water loss and contributed the optimization of plant water balance at water-deficient conditions. The drought provoked moderate and severe water stress in castor plants. Under stress conditions the leaf gas exchange parameters sharply decreased to preserve the castor photosynthetic apparatus from structural and functional damages. The enhanced leaf GPOD after 15 days of drought exposure indirectly indicated the presence of efficient control on the production of the active oxygen species. Subsequently, both photosynthetic pigments content and the apparent electron transport rate (ETR) were slightly diminished

and the maximal quantum yield of photosystem II (F_v/F_m) was not affected. Therefore, we may conclude that young castor plants are capable of efficient acclimation to drought. This observation confirmed previous reports concerning drought tolerance of this species and is a good initial point for further agronomic investigation for its behavior and productivity in rainfed conditions.

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ЖАС МАЙКЕНЕ (*RICINUS COMMUNIS* L.) ӨСІМДІГІНІҢ ҚҰРҒАҚШЫЛЫҚҚА ТӨЗІМДІЛІГІ, КЛИМАТТЫҚ ӨЗГЕРГІШТІГІ

Аннотация. Майкененің (*Ricinus communis* L.) Донская крупнолистная сорты Пловдив аграрлық университетінің (Болғария) жылыжайы жағдайында топырақ толтырылған ыдыстарда өсірілді. Жас өсімдік (4 жапырақ кезеңінде) келесі 15 күн ішінде топырақ ылғалдылығына байланысты үш түрлі жағдайда сыналды: оптимальді - 65-70% (бақылау), сонымен қатар минимум және максимум жағдайда. Осы мөлшерді ыдыстарды күнделікті таразыға өлшеу арқылы кстап отырдық. Физиологиялық және анатомиялық көрсеткіштері зерттеу жұмысының соңында өлшенді.

Құрғақшылық майкенеге әсерін тигізеді, оны барлық параметрлерінің өзгеруінен аңғардық.

Түйін сөздер: майкене, құрғақшылық, өсіру, жапырақ алақаны, фотосинтез.

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ЗАСУХОУСТОЙЧИВОСТЬ АКЛИМАТИЗАЦИОННЫЕ ИЗМЕНЕНИЯ В МОЛОДОМ РАСТЕНИИ КЛЕЩЕВИНЕ (*RICINUS COMMUNIS* L.)

Аннотация. Клещевина (*Ricinus communis* L.) сорта Донская крупнолистная была выращена в горшках с почвой в теплице аграрного университета г. Пловдив, Болгария. Молодые растения (4 листьев) проращивали в течение следующих 15 дней в трех разных режимах по влажности почвы: оптимальная - 65-70% (контрольный), а также почвы с дефицитом режимов влаги, провоцируя у растений стресс умеренной и тяжелой воды. Режимы воды были сохранены путем ежедневного взвешивания горшков и полив. Количество поливной воды для растений в оптимальных условиях водного режима был равен полному испарению с предыдущего дня (100% ПЭ), в то время как растения в дефицитных режимах были равны 75% (умеренный водный стресс) или 50% (дефицитом воды). Как физиологические, так и анатомические параметры были измерены в конце эксперимента. Клещевина реагируют на засуху, при развитии четко выделяются черты хемотрфис, что снизило потери воды и способствовали оптимизации водного баланса растения в воде-дефицитных состояниях. Засуха спровоцировала умеренной и тяжелой воды в касторовом растений, обозначается значительное усиление активности GPOD листьев. Сохранился фотосинтетического аппарата касторового растения в условиях засухи. Содержание фотосинтетических пигментов и явное электронного транспорта показатель (ЭТР) были немного уменьшены и максимального квантового выхода фотосистемы II (FV в/ФМ) не пострадали. Резкое снижение параметров листа газообмен в пострадавших от засухи растения можно рассматривать в качестве механизма, чтобы избежать возможных структурных и функциональных повреждений.

Ключевые слова: клещевина, засуха, выращивание, листовые пластинки, фотосинтез.