EFFECTS OF DROUGHT STRESS ON PHYSIOLOGICAL AND BIOCHEMICAL ADAPTATION RESPONSES IN YOUNG BLACK LOCUST *Robinia pseudoacacia* L. CLONES

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Black locust *Robinia pseudoacacia* L. is a fast growing tree, used in forest establishment. It is an economically important tree for tool production, obtaining timber and fuel as well as an important constituent element for landscapes. Though the tree is abundant, information on genetics, physiology, biology, wood quality and adaptability to different ecological conditions is limited and fragmented. The aim of this research was to study physiological and biochemical adaptation by comparing two black locust Clones that showed different responses to drought. The two Clones were exposed to different water regimes: Medium Stress and High Stress (50 and 25 % of water supplied to control). Physiological and biochemical measurements were made. Stress affected negatively the growth of both Clones. The effect of different stress intensity (MS and HS treatments) was observed on stem shape. When re-watered the stem showed partial recovery. Net photosynthesis rate in drought period did not show marked difference between the Clones. After soil rehydration both Clones recovered photosynthesis level. The Clones 1 did not show differences in osmotic potential when comparing Control and MS or HS trees. The Clone 2 showed increased osmotic potential in relation to stress intensity. The maximum potential in Clone 2 was comparable to the values for Clone 1. Amino acids, especially proline, increased in drought. The soluble sugars increased during the stress period in the stems of Clone 2. The increased absence of osmolytes increase in Clone 1 could be due to the absence of adaptive mechanism. Alternatively, osmolytes concentration was already too high to be increased more by drought. Different osmotic potential and changes during drought can be related to the growth during tree life. Selection of Clones according to osmolyte index could to select better trees for different climate zones.

Keywords: *osmotic potential, stem radial variation, soluble sugars, amino acids, soil water availability.*

Among the fast growing deciduous trees, black locust *Robinia pseudoacacia* L. is the third tree used in forest stand establishment and artificial reforestations after eucalyptus and hybrid poplars. The black locust is a medium-sized deciduous tree that commonly reaches 20 m as a single tree and 30 m within stands (Keresztesi, 1983; Huntley, 1990). It has a typical life span of about 60–100 years (Loehle, 1988), although the current longevity record in Europe is for more than 300 years (Peabody, 1982). Black locust is characterized by a dense and highly branched root system which enables it to successfully exploit nutrients and water resources of the soil, high vitality and seed production and excellent ability to fix free atmospheric nitrogen. As with other species of the Fabaceae family, the roots of black locust host rhizobia bacteria. These bacteria shows a capacity to intake nitrogen gas

from the air and convert it to a form usable by the plant. This process makes nitrogen available to other plants and improves the quality of the soils. Black locust can add between 23 to 300 kg of nitrogen per hectare every year (Cierjacks et al., 2013).

Black locust is abundant across Europe, occurring from Sicily in Italy to South Norway and from the Portugal up to the Caucasus (Vilà et al., 2009; Gederaas et al., 2012). Core areas of this species are in sub-Mediterranean to warm continental climates, where a rather high heat-sum is available and prolonged droughts are rare (Sitzia et al., 2016). The species was introduced in Europe in the early 17th century from Appalachian mountains (USA). In the late 18th and early 19th century extensive planting of black locust trees started in Central Europe. Currently it takes place in 42 European countries and the species is naturalised in 32 countries (Pyšek et al., 2009), covering with pure and mixed stands, for example 400.000 ha in Hungary, 200.000 ha in France, 250.000 ha in Romania and 230.000 ha in Italy (Rédei et al., 2011; Enescu and Dănescu, 2013; Sitzia et al., 2016). Its rapid spread has been promoted by increasing demands for the afforestation of neglected agricultural lands (Rédei, 1996; Rédei et al., 2014). In several countries it is an economically important tree and as a result *Robinia* has become a part of nature, landscape, culture and economics, including cultural and historical heritage. The yellow and greenish wood is durable, resistant to stem rot and insect damage, with a marked contrast of colour between young and mature wood. Black locust is highly appreciated as firewood because of its high calorific potential and high suckering capacity, which makes coppicing the most cost effective management system. The mechanical properties of the wood are moderate to high (Pollet et al., 2012; Cierjacks et al., 2013). The potential use includes fence posts, boatbuilding, flooring, mine timbers, turned objects, veneer and furniture. Moreover, it is a promising fast-growing tree species for biom ass production (Grünewald et al., 2009). Finally, black locust is a source of fragrant honey (Kenjerić et al., 2007), and its blossoms are used for cooking.

Nevertheless, the attempts to assign *Robinia* to the invasive alien species of EU cause public debates among various interest groups, and individual States may take measures against black locust mistakenly qualified as an invasive alien species (Vilà et al., 2009; Vítková et al., 2017).

In spite of its abundance, evidence on genetics, physiology and wood quality of black locust is limited. Some works refer to the most important vari-ables influencing black locust tree growth and wood formation: temperature, light, mineral nutrition, age (Hillinger et al., 1966; Keresztesi, 1988; Mebrahtu et al., 1993). A clonal selection initiated in the sixties in Hungary (Keresztesi, 1988) mostly aimed to improving wood production in black locust stands, but knowledge on genetic variability and adaptation of the selected material to unfavourable environmental conditions remains limited. Studies on the relationships between genetic polymorphism and phenotypic variation (Cheverud, 1984; Lande, 1984; Schlichting, 1986; Malvolti et al., 1994) may be useful for genetic improvement programmes. At present, few data are available on *Robinia* concerning relationships between physiological, biochemical, genetic traits and adaptability to different ecological conditions. Consequently, the cases of extending black locust stands to difficult areas and low amount of water due to poor soil quality have been few.

Water limitation and drought conditions are becoming quite common in many regions; climate models are predicting changes to precipitation distribution with summer rainfall decrease. Moreover, the intensification of extreme events will increse in the next few decades, affecting ecosystems, tree distribution, growth rate and productivity (Hickler et al., 2012). Planting black locust in the drier regions of central Europe (Rédei, 2002; Rédei et al., 2008; Grünewald et al., 2009) with annual mean precipitation lower than 600 mm · yr^{-1}, showed the potential of black locust to be relatively drought tolerant compared with other temperate deciduous tree species (Veste and Kriebitzsch, 2013; Mantovani et al., 2014*a, b*). Until now, most studies on black locust growing in drought conditions have focused mainly on ecophysiological adaptations of this tree (Zhang et al., 2012; Veste and Kriebitzsch, 2013), emphasising high plasticity of the species. To study the consequences of water shortage, a number of variables involved in the soil-plant-atmosphere interaction were analysed in field and under controlled environmental conditions. Increased drought may also indirectly reduce productivity via symbiotic nitrogen fixation, a key ecosystem process facilitated by plants such as black locust (Minucci et al., 2017). Thus, if drought negatively affected nitrogen fixation, it could reduce tree productivity. Plant nitrogen demand may have decreased if stomatal limitation on photosynthesis has lead to decreased growth. However, as soils rewet, nitrogen supply may increase as moisture limitation of mineralization and diffusion are alleviated. To maintain the integrity of carbon assimilation and

hydraulic systems, plants will undergo structural or physiological adjustment when they are confronted with soil moisture stress (Bréda et al., 2006). The effects of drought stress on the physiology of black locust, such as changes of the developed root systems, photosynthesis, stomatal control of transpiration and sap flow characteristics have been documented (Yan et al., 2010; Du et al., 2011). In recent years, plant water use has been measured using thermal dissipation probe methods (Du et al., 2011; Peng et al., 2015) that in areas subjected to drought are particularly suitable to investigate different strategies used by vegetation to cope with the limited availability of water in soil (Pataki et al., 2000). Therefore, it was hypothesized that the sap flow and growth of black locust would be inhibited due to the intensification of soil moisture stress (Zhang et al., 2018). Carbon starvation induced by drought stress was suggested (Parker et al., 1975), which is mainly caused by the reduction in photosynthesis, but with less decrease in respiration than photosynthesis leading to severe and eventually lethal decrease in the reserves of carbohydrates. However, there is a considerable variability and complexity in the response of carbohydrates to drought as well as in tree size, age, tissues, and species (Sala et al., 2012; Hartmann et al., 2013). Variations in carbohydrates concentrations resulting from drought stress in different black locust tissues may vary significantly (Zhang et al., 2015). Changes in carbon allocation, use, and transport have been observed to differ between above and below-ground tree tissues. H. Hartmann et al. (2013) reported that under drought conditions, carbohydrate concentrations were significantly reduced in roots, but not in leaves or branches.

A deeper understanding of the intertwined processes of black locust response to drought is still urgent, and needs further studies in order to identify growth performance and adaptation mechanism under different soil water regimes. The aim of this study is to cooperate to characterize black locust genotypes suitable for reforestation of degraded lands in unfavourable environments. Studies of drought effect on physiological and biochemical adaptation were conducted to compare two black locust clones that showed different responses to the environmental drought. Specifically, variation between water treatments and their effect on tree growth, osmolytes and their composition in different tree organs such as leaves, stem and roots were analysed. The study can provide information on the physiology and biochemical adaptation of black locust depending on soil water availability.

MATERIALS AND METHODS

Controlled pot experiments were conducted in central Italy (42°69′ N, 12°10′ E, 450 m AMSL). The climate of this region is warm continental with hot summers and cold winters. The site shows a mean annual rainfall of 850 mm, which mainly falls in spring and autumn, with June, July and August droughty, and a mean annual temperature of 13.9 °C.

Black locust mother trees of about 30–35 years old, growing in repository experimental station, were selected according to tree growth characteristics observed during several years.

In early March, from selected mother tree, root cuttings for clonal propagation were made to obtain saplings Clone 1 and Clone 2. The Clone 1 was considered more sensitive to drought than Clone 2, due to the reduced increases, full tree size and stem diameter, reported in the previous years during drought seasons. The root cuttings of selected tree clones were pot cultivated in 0.5 l containers with a mixture of sand and garden soil (2 : 1) in the greenhouse initially, and subsequently in the open field. In the middle of May, rooted cuttings were transplanted to 30 l pots containing a mixture of sand and garden soil (1 : 1). Plants were kept in the Institute experimental station to acclimate under no limiting water conditions. Once a week, plants were fertilised with a commercial liquid fertiliser added to the irrigation water. Plants were also preventively treated with chemicals against pest attacks and diseases. Later, in early July, the pots were placed in open field in rows 80 cm apart from the neighbours under full sunlight. They were kept well watered, soil was maintained at water field capacity. At the beginning of August, 70 trees of each clone were selected to the maximum possible uniformity in the stem height (1.0–1.2 m) and distributed randomly in the experimental area.

At the beginning of August, plants were divided into 3 groups such as Control (C), Medium Stress (MS) and High Stress (HS). MS and HS plants were provided with a rain shelter, which was wrapped on pot open topside in order to prevent the rain from interfering into watering regimes. C plants were kept well watered during the entire duration of the experiment. MS plants were watered with 50 % of the water supplied to C plants; and HS plants were provided with only 30 % of the water allowed for C plants. To determine the amount of water to be added, 4 pots of Control (for each Clone) were selected and weighed every 2–3 days, according to weather conditions, using an industrial balance with

Table 1. Scheduled time of experiment with three water regimes and correspondence between calendar and Julian days

Calendar day	Julian day	Treatment
March, 1–5	60–64	Harvest of root cuttings from selected trees
May, 14	134	1st selection of uniform sapling and transplant in larger pots
July, 1	182	Removal of all pots in open field
August, 1–2	213–214	Selection of trees and division into 3 groups (C, MS, HS)
August, 4	216	Start of drought treatment
August, 8–25	220–237	Three tree harvesting periods for laboratory analyses
September, 1	244	Re-watering all trees to Control level
September, 8	251	Harvesting trees for laboratory analyses

an accuracy of 5 g. After 28 days of drought stress all the trees were re-watered to C level. The experiment timing is summarized in Table 1.

Four trees of each treatment were used for all the experiment to evaluate weight and water to be added and to measure leaf gas exchange. Furthermore, to other 4 trees of each treatment the linear variable differential transducers (LVDT) were applied. During experimental period (beginning, during and at the end of drought and after re-watering) 4 trees of each treatment were removed to the laboratory de-potted, washed and used for laboratory analyses.

To evaluate stress level, leaf gas exchange (photosynthetic rate, transpiration rate, stomatal conductivity) was measured with an open system LCA3 (ADC, England). Measurements were made daily, before stress imposition, during the period of water stress treatment and one week after re-watering to follow the plants recovery.

Linear variable differential transducers (LVDTs) model DFg 2.5, (Solartron Metrology, Italy) were fixed on the tree stems. The LVDTs were connected to a datalogger with data being sampled every 15 minutes. LVDTs and datalogger resolutions give measurement accuracy equivalent to 0.1 mm of the tree stem movement. When necessary, the LVDTs can be repositioned without any disconnection of the device from the stem and interruption of data collection.

During the period of drought stress treatment the saplings were harvested for biochemical analyses. At each sampling time three plants for each treatment (C, MS and HS) were de-potted. The leaves, stems and roots were cut off and washed separately with distilled water. Any remaining surface water was removed with blotting paper, and all sample tissues were weighed and packed into labelled envelopes. Sampling was performed between 7 : 00 and 10 : 00 h. Within 2 h of collection, the samples were frozen. Subsequently, sample tissues were lyophilized. All dried samples were ground using

a grinding mill to be sufficiently fine to pass through a 40-mesh screen. Lyophilized materials were extracted with suitable solvent (water, 0.6 M $HClO_4$, ethanol) for the identification of osmolytes, soluble carbohydrates and amino acids determination by standard methods.

Total osmolytes were extracted in water of high purity and were measured with a cryoscopic osmometer (Osmomat 030, Gonotec, Germany). Free amino acid (extracted in 0.6 M $HClO_4$) was determined by acid ninhydrin method as described by S. Mapelli et al. (2001). Proline was assayed as described by L. S. Bates et al. (1973). Total soluble sugars and reducing sugars were extracted in 80 % ethanol at 80 °C and assayed respectively according to N. Nelson (1944) and M. Somogyi (1945) methods.

Lyophilized materials were useful for storage and to easy extraction with different solvents in precise concentration. However, taking into consideration that osmolytes exert their action and osmotic potential in watered tissues the data were recalculated on fresh weight basis.

Four trees were harvested at each time and individually analyzed with determination repeated 2–3 times. The data were elementary statistic, mean, and standard error (SE) was evaluated.

RESULTS AND DISCUSSION

The propagation of black locust through root cuttings gave satisfactory results both for Clone 1 and Clone 2, with a high number of uniform saplings. At the beginning of August, the plants were selected and randomly distributed in the experimental plot to be used for drought treatments begun on Julian day 216. Fig. 1 showed the increase of stem diameter. Control trees of both clones grew similarly during all the growing season, with growth decrease approaching in autumn (Julian day 264, first autumn day) and later stopped for winter rest.
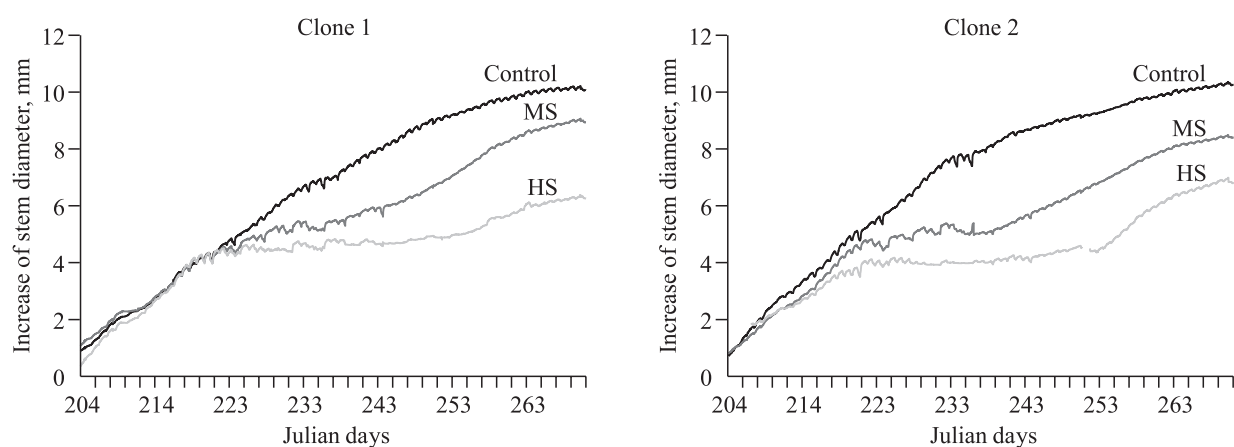
Fig. 1. Comparison of stem radial growth during growing season of the Clone 1 and Clone 2 subjected to different regimes: C = control; MS = moderate stress and HS = high stress. Drought began on Julian day 216 and terminated on Julian day 244 with soil re-watering.

Almost 10 days of water stress produced a negative effect on the growth of both black locust clones, with differentiation in stem dimensions between C plants and stressed plants (MS and HS treatment). The stem radial growth gave similar response for the two clones when drought was imposed. The effect of two different levels of replacing the water transpired by leaves was quite evident in radial stem size with different growth intensity, which was due to different soil drought levels.

If in MS trees slow growth was measured after about 20 days of water stress, the severe drought treatment (HS) totally blocked the stem growth in few days and remained blocked after the Julian day 244 when all the trees were re-watered and the stem of droughty trees showed a recovery of different amplitude in relation to the drought degree previously applied. The two clones evidenced marked differences in the rate of growth and sensitivity to the drought and Clones 2 subjected to HS recovered a higher stem size compared to Clone 1. This corresponds with the observation made during long period in experimental station which indicated that Clone 1 as more sensitive to drought than Clone 2, due to minor increasing and total size of trunk diameter. Thermal dissipation probe (TDP) used to evaluate drought, measured xylem sap flow that resulted from the limited amount of water and the black locust growth was inhibited due to the intensification of soil moisture stress (Peng et al., 2015; Zhang et al., 2018). The LVDT system used here directly measured the trunk size affected by the drought stress, which is certain to be caused by the amount of water absorbed and transported from roots. Furthermore, making use of LVDTs the phloem sap quantity and movement were evaluated through daily fluctuations of micro measurements performed with LVDTs.

With HS and stress period applied the trees produced wilted leaves (a few of them fell down), so the trees were able to recover growth after soil re-watering. In the other reported experiment, the soil drying was really severe and black locust trees did not survive (Zhang et al., 2018). The diameter of four trees for each Clone and water regimes were recorded; Fig. 1 reported only one tree for each treatment, as the patterns were similar.

Table 2 shows leave gas exchange, both net carbon dioxide and water vapour before drought stress application (day 216), during stress period (days 228 and 236), and a week after soil re-watering (day 251).

Control trees showed changes in net CO_2 assimilation, and smaller changes for water transpiration during the season. These differences could be related to temperature, sun light intensity or relative humidity differences in the day and time of measurement. Anyway, the effects of drought stress are quite evident. Carbon dioxide and water transpiration decrease as soil drying and time length increase. On Julian day 236 the CO_2 value became negative, which is a clear indication of the absence of photosynthesis but active respiration consumed some carbohydrate reserve in the trees. Surprising the values after soil re-watering were higher than those of Control trees. No clear explanation was evident but it is possible to hypothesize that during water stress the leaves stopped growing, or reduced, but after re-watering leaves could show higher stomatal density.

The drought and its intensity affected the trees growth, which was also suggested by the changes in

Table 2. Comparison of carbon dioxide and water transpiration in Clone 1 and Clone 2 subjected to drought stress and after soil re-watering. Drought period began at Julian day 216 and re-watering on Julian day 244

Clone	Stress level	Julian days			
		216	228	236	251
		Net CO$_2$ assimilation (A = µmol · m^{-2}· s^{-1})			
Clone 1	Control	17.61 ± 0.89	13.96 ± 1.67	6.18 ± 1.22	9.32 ± 1.27
	Medium Stress	15.98 ± 0.73	4.50 ± 3.62	−0.36 ± 0.22	14.25 ± 1.48
	High Stress	17.98 ± 0.69	2.03 ± 0.88	−0.47 ± 0.13	16.71 ± 2.03
Clone 2	Control	12.11 ± 0.62	13.40 ± 2.31	7.17 ± 0.90	12.25 ± 0.83
	Medium Stress	13.69 ± 1.25	5.30 ± 1.38	−0.55 ± 0.15	16.28 ± 1.33
	High Stress	12.99 ± 1.39	2.23 ± 0.66	−0.48 ± 0.12	16.71 ± 158
		Water transpiration (E = mol · m^{-2}· s^{-1})			
Clone 1	Control	3.80 ± 0.22	2.95 ± 0.46	2.26 ± 0.09	2.46 ± 0.38
	Medium Stress	3.30 ± 0.20	1.00 ± 0.85	0.32 ± 0.10	2.89 ± 0.38
	High Stress	3.69 ± 0.21	0.62 ± 0.19	0.03 ± 0.03	2.71 ± 0.40
Clone 2	Control	3.71 ± 0.28	2.90 ± 0.46	2.46 ± 0.25	3.29 ± 0.22
	Medium Stress	3.82 ± 0.26	0.85 ± 0.28	0.26 ± 0.08	3.72 ± 0.25
	High Stress	3.54 ± 0.52	0.49 ± 0.19	0.18 ± 0.08	3.40 ± 0.34

Table 3. Changes in fresh weight of plant organs during drought stress period and after re-watering ($n = 4$, ± SE)

| Clone	Stress level	Fresh weight, g			Leaves/roots
		Leaves	Stem	Roots	
		During drought stress, Julian day 228			
Clone 1	Control	19.33 ± 3.05	37.78 ± 6.83	142.55 ± 20.65	0.38
	Medium Stress	11.64 ± 3.92	23.36 ± 5.45	148.43 ± 26.15	0.23
	High Stress	8.23 ± 2.67	18.01 ± 3.83	121.77 ± 19.85	0.21
Clone 2	Control	21.70 ± 4.58	36.11 ± 5.03	154.88 ± 32.53	0.37
	Medium Stress	15.24 ± 3.09	31.86 ± 3.62	139.70 ± 36.86	0.33
	High Stress	11.54 ± 2.44	21.39 ± 2.16	129.83 ± 16.83	0.25
		After soil re-watering, Julian day 251			
Clone 1	Control	30.17 ± 5.72	47.58 ± 7.82	237.11 ± 31.64	0.32
	Medium Stress	14.53 ± 3.78	38.15 ± 6.48	200.23 ± 38.83	0.25
	High Stress	10.23 ± 1.27	36.96 ± 4.82	156.97 ± 30.56	0.28
Clone 2	Control	34.58 ± 4.98	50.54 ± 8.15	257.38 ± 42.78	0.33
	Medium Stress	16.31 ± 3.68	42.87 ± 6.83	189.45 ± 25.84	0.31
	High Stress	18.77 ± 4.72	39.08 ± 5.25	181.41 ± 27.23	0.31

fresh weight of tree organs presented in Table 3: all three organs (leaves, stem and roots) increased their weight less than the Control according to stress intensity.

Clone 1, again, proved to be more sensitive to the stress, which was suggested by the leaves/roots ratio that was more affected than in Clone 2 already at MS condition and lower recover of ratio was also measured on day 251 after a week of soil re-watering. As expected and described by J. M. Minucci et al. (2017), water stress and reduced weight also gave a reduction of root rhizobia. At the end of drought period (day 244) the rhizobia weight was 750–830 mg per root apparatus of C trees and decreased to 50–80 mg per root apparatus during MS and HS of both clones. Later in autumn about 2.5–3.2 g were weighed in C trees, 1.4–1.5 g for MS trees and 0.8–1.0 g for HS trees.

The total osmolyte concentrations in tree organs of Clone 1 and Clone 2 were shown and compared in Fig. 2.

The leaves and root of Clone 1 did not show evident differences in osmolyte quantities when comparing Control to MS and HS trees. In this Clone 1 a modest increase, in absolute value in comparison to the higher value for leaves and roots, was measured in the stem, but it is sufficient to distinguish C, MS and HS stems. The Clone 2 samples showed the osmolyte increases in leaves and roots as stress intensity increased. Stems of Clone 2 showed os-
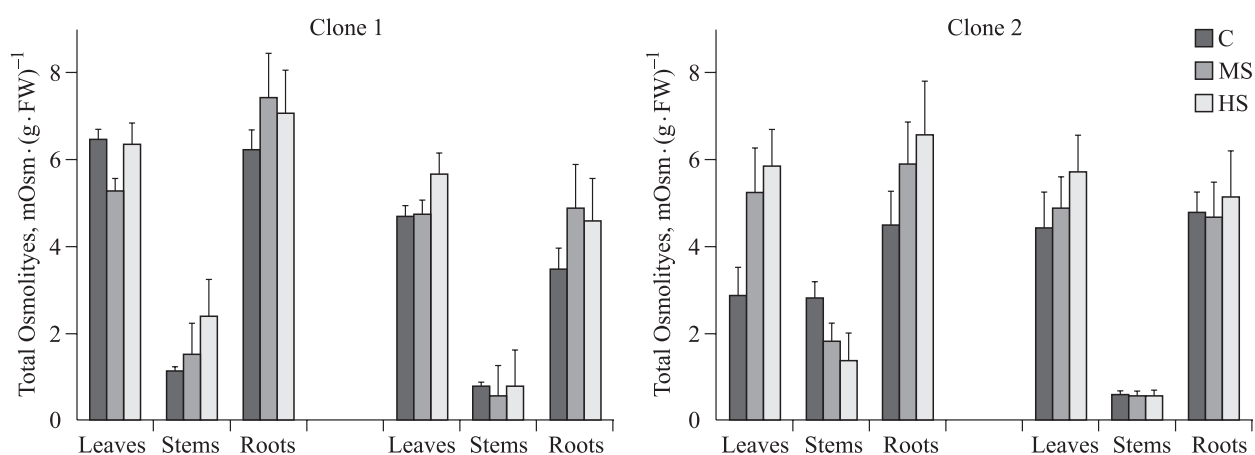
Fig. 2. Changes in osmolyte concentration in plant organs (leaves, stem and roots) in the two Clones of black locust control trees (C) or subjected to medium (MS) or high (HS) soil drought condition. Left part of figures for the samples on Julian day 228, twelve days after the beginning of stress; right part of figures on Julian day 251, seven days after soil re-watering. (n = 4 trees, analyses repeated 2–3 times for each sample, mean value ± SE).
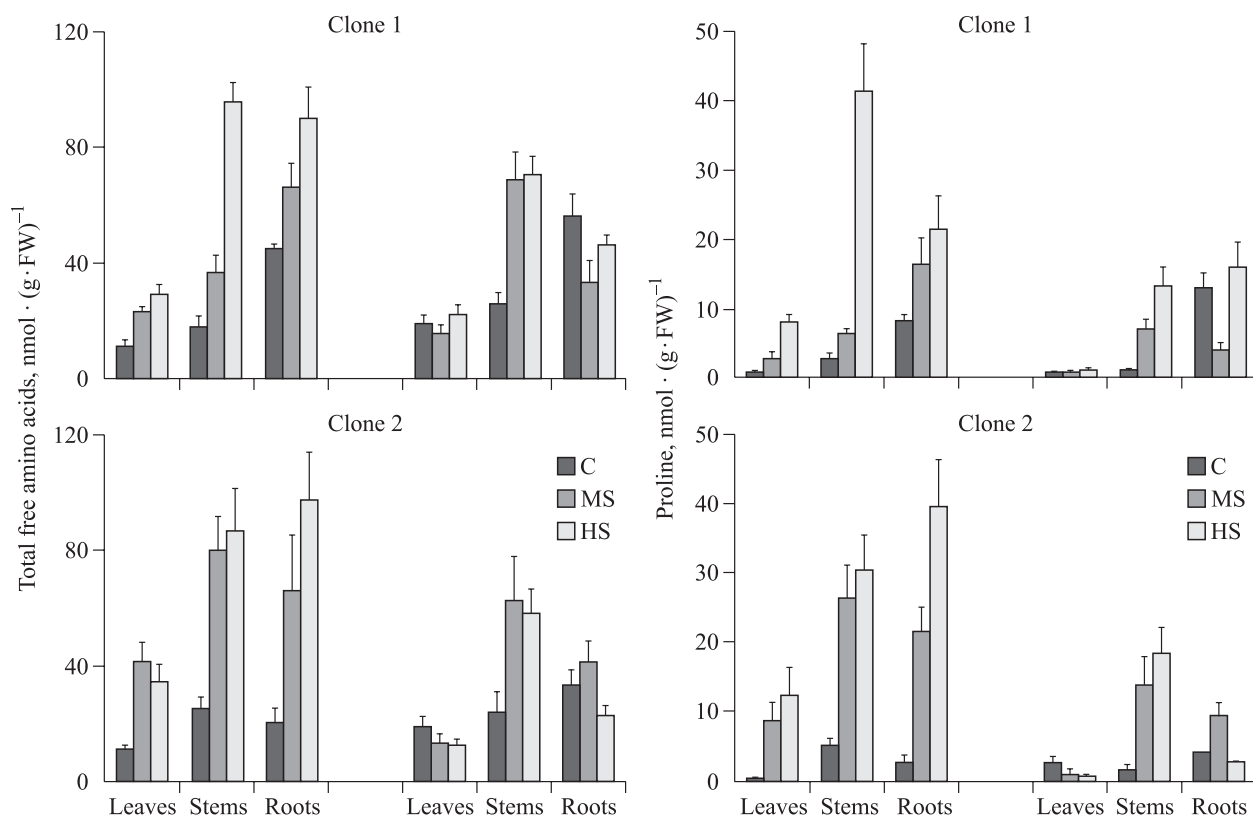

Fig. 3. Changes in total amino acid concentration (left panels) or proline (right panels) in the two black locust Clones subjected to different water regimes (control – C; medium stress – MS; high stress – HS). For further details, see legends in Fig. 2.

molytes decrease. It is interesting to note that the maximum osmolyte concentration measured in Clone 2 drought leaves and roots was comparable to the osmolyte values measured in leaves and roots of Control Clone 1. The absence of total osmolytes increase in stressed Clone 1 could be due either to the absence of adaptive mechanism or the high osmolyte concentration in C samples, for the concen-

tration was already too high to be increased more by drought stress condition.

The analyses of the substances that are part of the osmolyte components were carried out to study free amino acids and soluble carbohydrates as the more common and plentiful components. In both clones the determination of total amino acids showed increased concentration due to drought (Fig. 3).
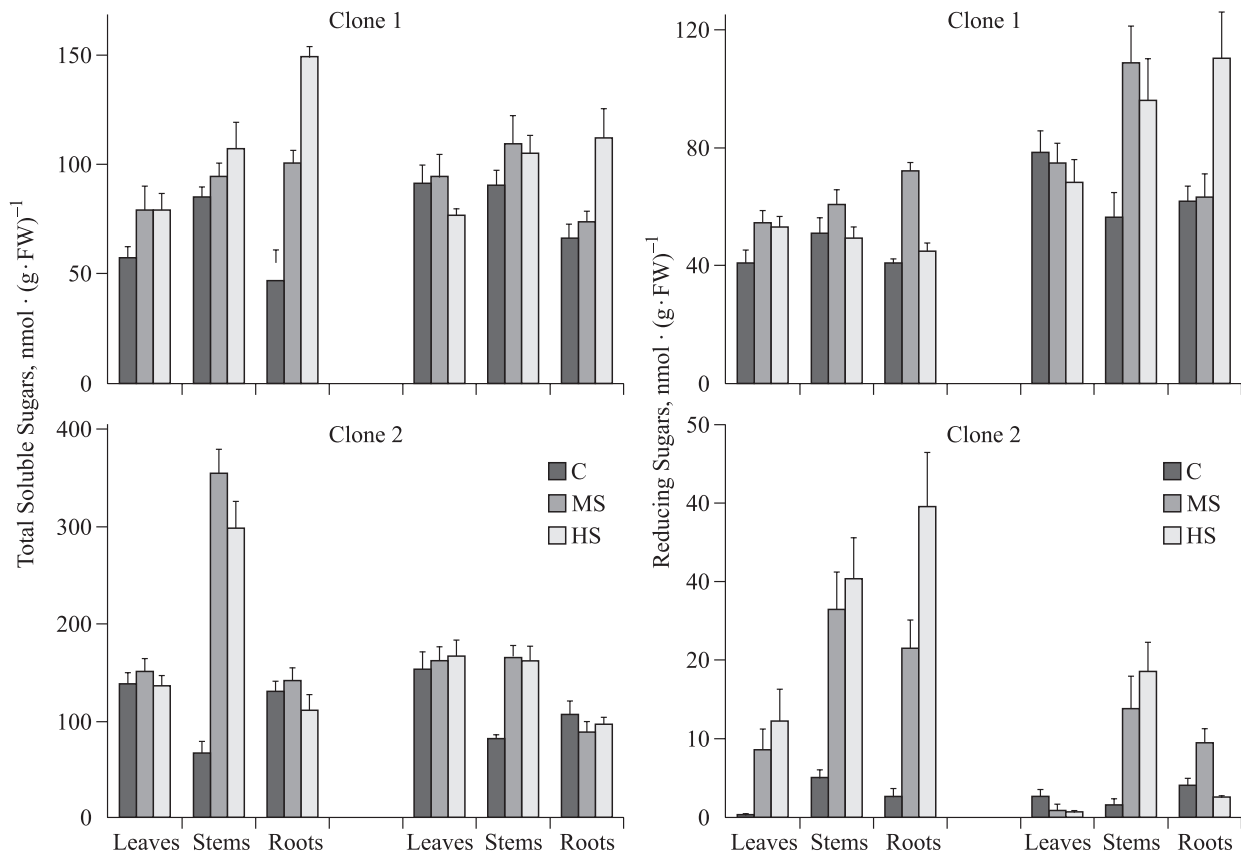
Fig. 4. Level of total soluble sugars (left panels) and reducing sugar (right panels) in control (C) and the two levels (MS, HS) of drought stressed *Robinia* trees of the two Clones studied and after 28 days of different intensity of drought treatment. For further details, see legends in Fig. 2.

Furthermore, the proline, generally considered a multifunctional amino acid and a stress marker (Kuznetsov and Shevyakova, 1999; Szabados and Savoure, 2010) also showed the increase of its content in both clones during drought period (Fig. 3). These data indicate that both clones reacted to stress increasing amino acids concentration, with proline increasing up to 50 % of the total amount of free amino acids. Main amino acids and proline increases were detected in roots and stem. After soil re-watering the trend was to revert with the reduction of the concentrations. The amino acids and proline concentrations were comparable for both clones.

The determination of total soluble sugars and reducing sugars indicate general and significant differences between Clone 1 and Clone 2 both during drought stress and after soil re-watering (Fig. 4).

Clone 1 showed an increase of total sugars during the stress period quite evident in roots. One more point to note for Clone 1 was that total sugars were almost in large part due to reducing sugars, which increased more after soil re-watering. In Clone 2 during MS and HS period a dramatic increase of total soluble sugars was measured in stem, up to or more than 300 mmol/g of fresh weight. This allowed to assume a fast solubilisation and mobilization of storage reserves in roots and stems. In Clone 2 the reducing sugars showed a pattern related to stress intensity quite evident for stems and roots (Fig. 4).

In Clone 2 the reducing sugars concentration was lower than in Clone 1 and more significant was the concentration decrease after re-watering. A role of carbohydrates in plants and the relation to abiotic stress are well known (Halford et al., 2011; Khan and Naqvi, 2012). Also carbohydrates changes in black locust were recently studied, exciting interest to soil drying effect on black locust growth (Zhang el al., 2015). The results indicate that carbohydrates may have produced a stronger influence than amino acids do on the black locust capacity to adapt to water stress.

However, the data on analyzed osmolytes did not justify the higher osmolytes concentration (Fig. 2) present in Clone 1 in well watered conditions. If the osmolyte adjustment was important and played a significant role in more water stress resistant clones, the kind of osmolyte substances already present in well watered trees but less adaptable to drought must be studied.

CONCLUSION

The data indicate that different osmolyte concentrations and variable capability to adapt during drought condition can be one of the causes of the differences in trunk growth and accumulation of wood during tree life. However, the analyses carried out did not explain what are the components that give the high osmotic potential measured in control trees of Clone 1 and the modification occurring in Clone 2 during drought period. Further analysis will be developed considering other carbohydrates (fructan, trehalose), organic acids or polyphenols as possible osmolytes. Preliminary test on other black locust clones that showed different growth under water stress conditions suggested osmolytes as a possible adaptation mechanism. Selection of clones by osmolyte content index could help to identify the better clone for different environmental climate zones. The results provide information, which can contribute to genotypes selection and management strategies for new plantations. In addition, it can suggest new studies on the relationships between genetic background and biochemistry of black locust.

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ФИЗИОЛОГИЧЕСКАЯ И БИОХИМИЧЕСКАЯ АДАПТАЦИЯ МОЛОДЫХ КЛОНОВ РОБИНИИ ЛОЖНОАКАЦИЕВОЙ *Robinia pseudoacacia* L. К СТРЕССУ, ВЫЗВАННОМУ ЗАСУХОЙ

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Робиния ложноакациевая *Robinia pseudoacacia* L. – это быстрорастущие деревья, используемые в лесном хозяйстве. Применяются в производстве инструмента, топлива и столбов, а также при создании ландшафтов. Несмотря на широкое распространение, знания об этих деревьях из области генетики, физиологии, биологии, качества древесины и способности адаптироваться к различным экологическим условиям ограниченны и фрагментированны. Цель данного исследования – изучение физиологической и биохимической адаптации двух клонов робинии ложноакациевой с разной реакцией на засуху. Два клона были подвергнуты различным водным режимам: среднему и сильному стрессу (50 и 25 % от количества воды в контроле). Проведены физиологические и биохимические измерения. Стресс оказал отрицательное влияние на рост обоих клонов. Очевидным было влияние стресса различной интенсивности (среднего и сильного) на форму ствола. При повторном поливе ствол показал частичное восстановление. Не выявлено существенной разницы между клонами по чистой скорости фотосинтеза в период засухи. После регидратации почвы оба клона восстановили уровень фотосинтеза. Клоны 1 не показали различий в осмотическом потенциале по сравнению с контролем и деревьями, испытывающими средний и сильный стресс. Клон 2 характеризовался повышенным осмотическим потенциалом по отношению к интенсивности стресса. Максимальный потенциал в клоне 2 был сопоставим со значениями в клоне 1. Содержание аминокислот, особенно пролина, увеличилось при засухе. Содержание растворимых сахаров в стволе клона 2 возросло в период стресса. Отсутствие увеличения содержания осмолитов в клоне 1 может быть связано с отсутствием адаптивного механизма или с тем, что концентрация осмолитов уже была слишком высока, чтобы ее можно было увеличить в результате засухи. Различный осмотический потенциал и изменения во время засухи могут быть связаны с ростом дерева на протяжении жизни. Выбор клонов по индексу осмолита позволяет выбрать лучший клон для разных климатических зон.

Ключевые слова: *осмотический потенциал, радиальная изменчивость ствола, растворимые сахара, аминокислоты, доступность почвенной воды.*