Influence of Water Deficit Stress on Some Physio-morphological Indicies at Some Oilseed Rape Cultivars

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This study demonstrates that the water deficit stress had a negative influence on the studied morphological and physiological traits. The results of this study gave useful information of the studied cultivars regarding their response to the drought stress. The experiment was made in green house conditions at the University of Agricultural Sciences and Veterinary Medicine Ion Ionescu de la Brad Iassy, in the year 2013-2014. The comparison between the studied cultivars and the water deficit stress levels showed significant difference between genotypes at different water stress levels. The plant height is given by the genetic characteristics but is often influenced by the environmental conditions.

Keywords: oilseed rape, cultivars, water deficit stress, gas exchange

Oilseed rape (*Brassica napus* L) is considered to be a plant with high economic importance. During the last years, this crop has become a significant important crop for agriculture and now is the third source in the world of vegetable oil after soybean and palm [1]. Rapeseed oil has many potential uses other than as oil for nutrition [2, 3]. Beside its uses in human nutrition and industry, rapeseed oil is currently use for the biodiesel production. This kind of fuel has some advantages over other fuels because it produces less smoke, have a higher octane value, produces lower carbon monoxide and hydrocarbon emissions, and are biodegradable and non-toxic [4]. For this reasons the areas cultivated with oilseed rape started to be higher in Europe.

A major problem in the cultivation of oilseed rape in some countries, especially in Romania is the water deficit stress. This stress is considered to be one of the most important stresses of agricultural plants, affecting the plant growth and the yield quality and quantity [5]. The water deficit in plants can produce some physiologic disorders like reduce level of the photosynthesis and transpiration [6, 7]. Besides these disorders, previous studies reported that at oilseed rape, drought reduces the biomass and seed yield, harvest index, plant height number of silique and seed, seed weight and days to maturity [8-10]. For this reasons, the aim of the breeders is to breed for some resistant oilseed rape cultivars with drought resistance.

At the biochemical level, the generation of the reactive oxygen species (ROS) is the response of the eukaryotic cells to the drought stress. The production of ROS in plants is known as oxidative burst and is an early reaction of the plant defense to water stress. In the normal processes in the plant, the ROS which includes oxygen ions, free radicals and peroxides are a result of the metabolism of the plant and have an important role in cell signaling. The ROS level increase very much during the drought stress producing a oxidative damage to proteins, DNA and lipids. Because its highly reactive, the ROS can damage plants by increasing lipid peroxidation, protein degradation, DNA fragmentation and ultimately cell death.

The drought stress determines the formation of the oxidative stress in plants by generation of the reactive oxygen species (ROS). The membrane lipids can be directly attacked by the ROS such as O_2 - H_2O_2 and •OH radicals producing the increase the lipid peroxidation. The increase of the production of ROS under the drought stress, increases the content of malondialdehyde (MDA). The content of this is considered to be an indicator of oxidative damage. A suitable marker for membrane lipid peroxidation is the MDA content. The decrease of the membrane stability determines a increase of lipid peroxidation caused by ROS [11-20].

The aim of this study was to evaluate the main traits of some oilseed rape cultivars for the water deficit tolerance in order to determine the tolerant and sensitive cultivars.

Experimental part

Materials and methods

The experiment was made in green house conditions at the University of Agricultural Sciences and Veterinary Medicine Ion Ionescu de la Brad Iassy, in the year 2013-2014. The growth conditions for the plants were a temperature regime of 22 – 24^{0}C during the day and 15-17^0 in the night with a photoperiod of 10/14 hours. The humidity was of 50-60%.

Chemical composition

In function of variety and vegetation conditions, the chemical compositions of seeds are characterized by a content of:

- 33-49% fat
- 19-20% crude protein
- 17-18% non-nitrogenous extracts.

The content of colza rape seeds in oil pass by 40% at available varieties from erucic acid (type "0"). In comparative crops at ICCPT Fundulea, the oil content is between 43.8 and 47.2%.

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Table 1
DETAILS ABOUT THE STUDIED OILSEED RAPE CULTIVARS

No.	Cultivar	Origin	Type
1.	Libritta	Germany	Winter oilseed rape
2.	Lingot	France	Winter oilseed rape
3.	Tapidor	France	Winter oilseed rape
4.	Buko	-	Winter oilseed rape
5.	Bridger	SUA	Winter oilseed rape
6.	Capricorn	Great Britain	Winter oilseed rape
7.	Libraska	Germany	Winter oilseed rape
8.	Collo	Germany	Winter oilseed rape
9.	Silesia	Czechoslovakia	Winter oilseed rape
10.	Enroll	France	Winter oilseed rape
11.	Silvia	Germany	Winter oilseed rape
12.	Tamara	Germany	Winter oilseed rape

Table 2
ANALYSIS OF VARIANCE AT THE RAPESEED TRAITS UNDER THE WATER DEFICIT STRESS

Source		Mean Squares									
SV	df	PH	NP	NB	NSS	SL	YP	GW	SC	NP	CO_2
Cultivars	11	30350777,88*	2422451,36*	1187,78*	12036,44*	34,151	82629,73*	28,498*	0,067	267,79	0,435
Irigation level	3	20743398,83*	2222451,36*	283,650	1277,939*	211,562*	7704,037 *	2,715	2,519*	6531,155*	4,056*
Cultivars *Irigation levels	33	35725145,70*	18130,081	2,333^{ns}	38,840^{ns}	0,376^{ns}	2921,20*	0,032^{ns}	0,005	12,813^{ns}	0,075^{ns}
Error	432	34373810,22	1187,781	1,444	11,720	0,008	0,566	0,123	0,0001	0,435	0,088
CV		11,924	4,91	3,04	6,30	6,30	8,88	1,73	4,09	3,49	3,49

Ns and * non significant and significant at 05% level

At available varieties from erucic acid and glucosinolates (type ”00”), the oil content was between 43.8 and 47.2%.

At varieties cultivated in our country, the oil content in grains is between 44.5 and 45.8%. Generally, the oil content at colza grains is between 43 and 48%.

Biological material

The biological material for this study comprised 15 genotypes of rapeseed cultivars proceeded from the Centre for Genetic Resources Netherlands. Details regarding the studied genotypes can be observed in table 1.

First, the plants were cultivated in small pods of 5x5 cm a mixture of sand, peat and compost (1:1:2) for the vernalization. The vernalization was performed in a climatic chamber RUMED 4021. After the vernalization, the plants were transferred in 10 kilogram vegetation pots in the same soil mixture and moved in the green house. The water deficit stress was applied to each cultivar from the stem elongation to physiological maturity. The experiment was made with three irrigation levels: control (100 % FC), well watered stress (75 % FC), mild watered stress (50% FC) and severe stress (25% FC). In order to evaluate the influence of the irrigation levels upon the plants development, at the end of the vegetation stage of the studied cultivars some morphological traits were measured such as: plant height (PH), number of pods per plant (NP), number of branches per plant (NB), number of seeds per pods (NSS), pod length (SL), yield per plant (YP) and 1000 grain-weight (GW). The stomatal conductance (SC), gas exchange(CO_2) and the intensity of the photosynthesis (NP) were measured for each cultivar at each irrigation level five days from late flowering until leaf senescence using a portable photosynthesis system LCI PRO from ADC BioscientificLdt.

Statistical evaluation was performed using the XLSTAT and SPSS v.13 software package.

Results and discussions

The analysis of variance showed significant difference on all of the traits between the different irrigation levels. It can be observed that different irrigation levels on the studied cultivars had different influence on the studied traits (table 2). The interaction between cultivar *irrigation level was significant only at plant height, yield per plant, stomatal conductance, net photosynthesis and the CO_2 concentration (table 2).

The comparison between the studied cultivars and the water deficit stress levels (table 3) showed significant difference between genotypes at different water stress levels. The plant height is given by the genetic characteristics but is often influenced by the environmental conditions [11]. In this experiment, the highest value of the plant height (110 cm) was obtained in the irrigated (control) variant and applying the water stress decreased with 60 % (table 3). The reduction of the plant height can be caused by the reduction area of photosynthesis which

Table 3
COMPARED MEANS BETWEEN GENOTYPES IN DIFFERENT WATER STRESS LEVELS

Treatment	PH	NP	NB	NSS	SL	YP	GW	SC	NP	CO_2
Control	110.91*	595.46*	9.00*	31.21*	7.37*	79.59*	4.95*	0.53*	28.04*	0.73*
75	89.91*	535.85	7.28	28.91*	7.22	71.69	5.06*	0.33*	23.53*	0.59
50	77.83	476.46	6.20	25.20	6.18*	64.09	5.20*	0.24	16.99*	0.48
25	66.59*	423.08*	5.46*	24.19*	4.49*	61.96*	5.29*	0.21*	11.22*	0.29*
Cultivars										
Libritta	75.898*	464.922*	6.275	79.247*	7.006*	72.103*	5.315*	0.340*	24.151*	0.484
Lingot	87.561*	456.585*	5.350*	20.583*	4.860*	33.916*	4.836*	0.360*	17.772*	0.489
Tapidor	86.050*	238.389*	5.850	23.237*	6.190*	33.576*	4.353*	0.292*	22.050*	0.545
Buko	78.317*	247.199*	8.975*	11.807*	7.129*	14.224*	4.526*	0.364*	21.913*	0.493
Bridger	76.998*	380.514*	6.100	12.150*	5.350*	30.974*	4.206*	0.386*	21.524*	0.451
Capricorn	91.499*	193.424*	6.650	24.975	6.953*	26.938*	5.466*	0.335*	16.517*	0.513
Libraska	97.958*	1090.917*	8.125*	26.905*	6.794*	170.996*	5.082	0.318*	17.770*	0.542
Collo	98.364*	609.586*	8.075*	24.767	7.795*	88.716*	4.994	0.309	22.232*	0.476
Silesia	84.625*	668.308*	8.525*	22.055*	5.608*	122.252*	7.522*	0.294*	20.145	0.544
Enroll	57.173*	677.512*	6.550	32.600*	5.728*	85.659*	5.044	0.372*	17.380*	0.696*
Silvia	107.315*	533.592	7.175*	23.835*	7.038*	75.590*	5.180*	0.240*	16.691*	0.544
Tamara	93.951*	531.617	6.150	26.401	5.317*	77.058*	4.955	0.332*	21.176*	0.495

Based on observed means. *The mean difference is significant at the .05 level

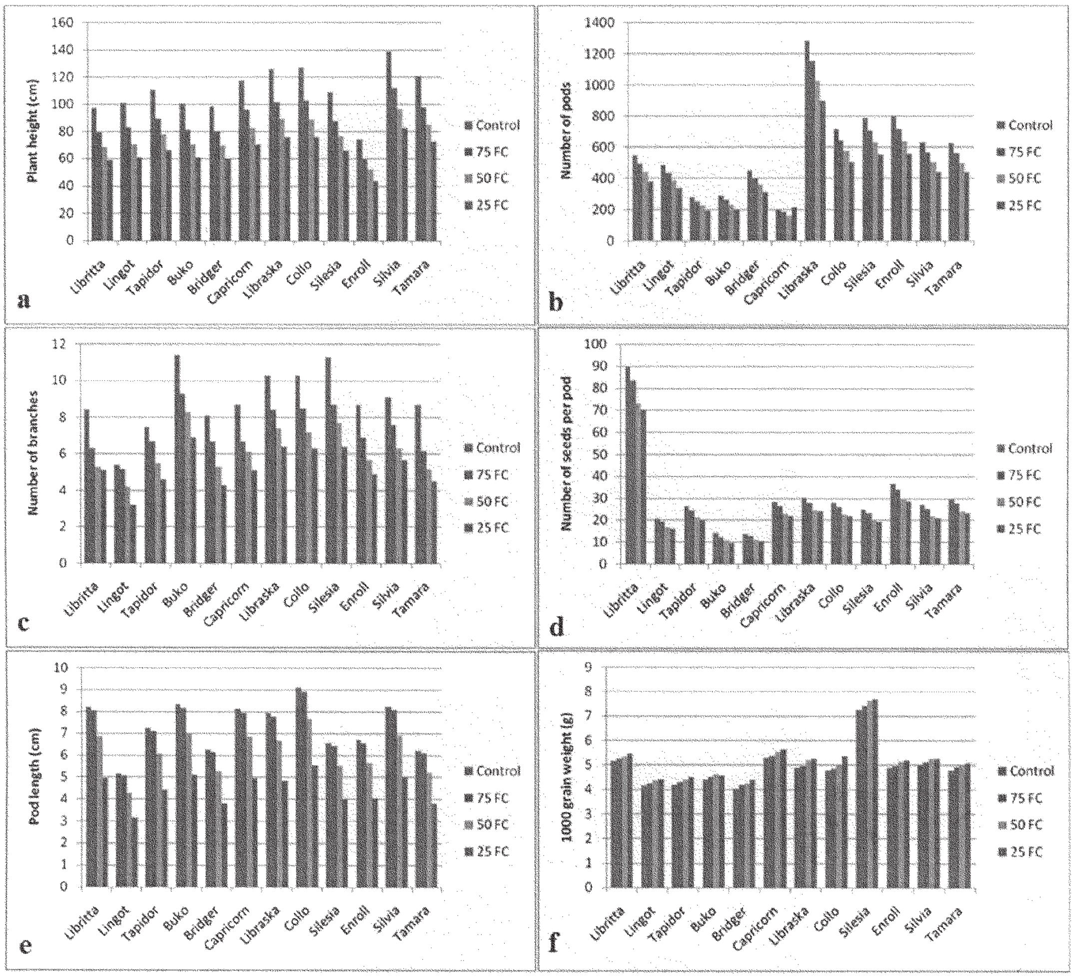

Fig. 1. Interaction between the studied cultivars and water deficit stress levels: a – Plant height, b – number of pods, c – number of branches, d – number of seeds per pod, e – pod length, f – grain weight

produces a decrease of the chlorophyll content that rinse the energy consumed by the plant to take the water and to increase the protoplasm density and to change the respiratory paths and the activation of the phosphate penthouse, etc. [12, 13].

The biggest value of the plant height was recorded at “Silvia” cultivar of 107.35 cm and the smallest plant height was at “Bridger ” of 76.99 cm. The number of pods per plant is known to be a major element in determining the yield in brassica species. This element is influenced by some factors like variety, inheritance and environment conditions. The highest number of pods was obtaining at “Libraska” cultivar in the control condition and the smallest number of pods was at “Tapidor” at 25% FC (fig. 1). It can be observed that the number of pods decreased with 71% in the highest level of water stress of 25% FC. Regarding this trait [14] reported a 59% decrease of the number of pods in some studied rapeseed cultivars exposed to water deficit stress.

Some authors, in their studies affirm that at *Brassica* species, the genotypes with a high number of pods per plant will give more seed and more oil [15-17]. In case of these experiment, it can be observed that the highest amount of number of pods were obtained at “Libraska” cultivar followed by “Silesia” and “Enroll” these cultivars having also the biggest amount of yield per plant (fig. 1).

The yield per plant differed significantly among the tested cultivars. Some researchers reported that the reduction of the yield per plant can be determined by the level of the water deficit stress and affects also the other yield components such pod per plant, seed per pod and 1000 grain weight [18-20]. It can be observed that in our experiment the yield per plant decreased at all the genotypes with the increase of the water deficit stress (fig. 1). Similar results were obtained by Khalli et al. [21] when evaluated the response of some spring canola genotypes at the water deficit stress. The highest values of yield per plant (g) was observed at „Libraska” and “Silesia” cultivars in both control and water stress levels and the smaller value for this was at „Buko” cultivar. The water deficit stress reduced all the morphological traits (fig. 1). The 100 grain weight had the biggest value at “Silesia” cultivar at 50 % FC and the smallest value at “Bridger” cultivar in the control variant.

In the normally process of the photosynthesis there are two reactions: the light reactions which happens during the day when the light energy is converted ATP and NADPH and oxygen is released, and dark reactions, in which CO_2 is fixed into carbohydrates by utilizing the products of light reactions, ATP and NADPH [22-24]. The general equation for photosynthesis is therefore: $2n\ CO_2 + 2n\ DH_2$ + photons $\rightarrow 2(CH_2O)n + 2n\ DO$ (Carbon dioxide + electron donor + light energy $\rightarrow$ carbohydrate + oxidized electron donor). In the case of drought stress, the photosynthesis is affected [19]. The effect of the drought upon the photosynthesis is expressed by the decrease of the CO_2 availability caused by diffusion limitations through the stomata and the mesophyll [25, 26] or the alterations of photosynthetic metabolism [22, 23] or they can arise as secondary effects, namely oxidative stress. In order to resist to the drought stress, plants have developed some strategies to resist to this stress. One of these strategies of the plants is the stomata closing during the water deficit stress. These adaptations have as effect the accumulation of the CO_2 which diminish the photosynthesis [17, 18]. Regarding this experiment, it can be observed that drought stress had significant effect on the stomatal conductance, net photosynthesis and intercellular CO_2 concentration (table

Table 4
MATRIX OF SIMPLE CORRELATION COEFFICIENTS (r) FOR THE ESTIMATED TRAITS IN RAPESEED CULTIVARS

	YP	PH	NP	NB	NSS	SL	GW	SC	NP	CO_2
YP	1									
PH	0.264**	1								
NP	0.934**	0.293**	1							
NB	0.318**	0.549**	0.329**	1						
NSS	0.218**	-0.005	0.147**	-0.019	1					
SL	0.170**	0.673**	0.189**	0.610**	0.256**	1				
GW	0.421**	-0.074	0.227**	0.107*	0.116*	-0.136**	1			
SC	0.037	0.585**	0.178**	0.543**	0.140**	0.490**	-0.222**	1		
NP	0.075	0.657**	0.150**	0.579**	0.267	0.725**	-0.188**	0.807**	1	
CO_2	0.128**	0.305**	0.180**	0.305**	0.105*	0.343**	-0.052	0.366**	0.400	1

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

2). The stomatal conductance decreased with the level of stress, having the smallest value (0.21) at 25% FC determining also the decrease of the net photosynthesis. The results from this study indicated that the drought stress decreases the stomatal conductance, the CO_2 concentrate and the net photosynthesis (table 3). The smallest value of the SC, NP and CO_2 was observed at the 25% FC and the biggest value was in the control variant. These results agree with the results obtained by Naderikharajii [27] in a study where they evaluated the effect of drought stress on photosynthetic rate of some rapeseed cultivars they obtained a value of 0.29 of the stomatal conductance under the water deficit stress. It can be observed that under water deficit stress, the plant is adapting to the stress by closing the somata leading to a decrease of absorbing the CO_2 and of the net photosynthesis. It seems like the plants tend to consume a lot of energy to absorb the water which cause a reduction of the production of the photosynthetic assimilates [12].

The correlations among the measured traits are presented in table 4. It can be observed that there is a strong correlation between the plant height, number of branches, number of pods, and number of seed per pods, pods length and 100 grain weight with the seed yield per plant. The highest correlation was observed between number of pods and yield per plant ($r=0.934$, $P<0.01$). Similar results were obtained by Shirani [11] which found also significant correlation between number of pods and seed yield. Also it can be seen a significant correlation between 100 grain weight and see yield ($r=0.421$, $P<0.01$). The number of pods per plant and number of branches correlated positively ($r=0.329$, $P<0.01$), this results according with Sadaqat [28] that found a significant positive correlation between pods per plant and number of branches per plant. These results showed that among the yield components the number of pods per plant and 1000 grain weight had the greatest influence on the seed yield. These results are similar with other researches and who analyzed soil water deficit [13, 15, 16, 29-33].

The cluster analysis based on the studied traits under the drought and normal conditions grouped in three clusters. In the first cluster is only the "Libraska cultivar" which is characterized by the biggest yield production, the biggest number of pods per plant and the biggest value of the plant height in all the variants (Control and stress). The second cluster grouped eight cultivars with mean values of the studied traits and the last cluster grouped three cultivars with the smallest value of seed yield, number of pods and 100 grain weight.

Conclusions

This study demonstrates that the water deficit stress had a negative influence on the studied morphological and physiological traits. It was observed a different response of the cultivars to imposed water stress condition that determined the drought tolerance ability of the studied rapeseed cultivars. Among the studied cultivars "Libraska" proved to be the most resistant to drought conditions and had the least reduction of the yield compared with the other studied cultivars. Also the cultivars "Collo", "Silesia" and "Eroll" showed a good tolerance to the drought having good values of the studied yield component in the drought conditions. The results of this study gave some useful information of the studied cultivars regarding their response to the drought stress. This information could be used in the future studies in the rapeseed breeding programs for obtaining some new hybrids with improved resistance to drought.

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