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DROUGHT STRESS INFLUENCE ON PEA PLANTS (*PISUM SATIVUM* L.)

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Abstract:

Pisum sativum L. (garden peas) are highly nutritious vegetable crops destined for human consumption. Pea seeds are a valuable source of protein (23–25 %), slowly digestible starch (50 %), soluble carbohydrates (5 %), insoluble fibers, and important sources of vitamins and minerals. Apart from the genetic (variety) differences, the chemical content of pea seeds is influenced by environmental factors (cultivation area, soil composition and characteristics, trace elements and minerals, total rainfall or lack of rain, relative humidity, solarization, average temperatures, etc.).

In this study, we analyzed the drought stress on Petit Provencal, a commonly used garden pea variety in Romania. In the case of drought stress, the plants suppress stomatal opening to conserve water. During this experiment, the plant photosynthetic parameters as assimilation rate and stomatal conductance have been decreased linearly with soil water capacity. The light dependence curves of photosynthetic parameters have been shown a sharp decrease at low soil water content. The recovery parameters achieved the same values after seven days of normal watering. The polyphenols content of the seeds does not vary significantly for plants normally watered compared to those kept in drought stress

Keywords: *Pisum sativum* L., drought stress, phenolic content, photosynthesis.

Introduction

Pisum sativum L. (garden peas) are highly nutritive vegetable crops destined for human consumption widely cultivated alongside common beans. Pea seeds are a valuable source of protein (23–25 %), slowly digestible starch (50 %), soluble carbohydrates (5 %), insoluble fibers, and important sources of vitamins and minerals (Cervenski et al., 2017; Zilani et al., 2017).

Potential health-promoting properties are correlated with pea consumption, including laxative and anti-diabetic effects, alongside cardioprotective, anti-obesity, antioxidant, antifungal, antimicrobial and anti-tumor properties. Most of these health benefits are attributed to their high nutritional properties, in particular, to their chemical composition of essential amino acids (methionine, cysteine, lysine, threonine and tryptophan), carbohydrates (starches and fibers), minerals, vitamins, fatty acids (omega 9, omega 6 and omega 3), and valuable amounts of bioactive compounds such as polyphenols (Mejri et al., 2019; Nithiyanantham et al., 2012).

Almost all plants (fruits, vegetables, seeds) contain naturally occurring compounds like polyphenols, making them important sources of antioxidants with many potential health benefits (Tanase et al., 2019).

Apart from the genetic (variety) differences, the chemical content of pea seeds is influenced by environmental factors (cultivation area, soil composition and characteristics, trace elements and minerals, total rainfall or lack of rain, relative humidity, solarization, average temperatures, etc.) (Cervenski et al., 2017; Zilani et al., 2017).

Harsh weather conditions (drought) especially in the flowering and pod filling stages, have a negative influence against pea crops yields and can modify their protein and starch ratios (Baigorri et al., 1999; Cervenski et al., 2017).

Soil water content (drought) needs a few days to induce stress in plants, however, in nature drought is usually associated with high temperatures, resulting in double stress factors for the plants. During drought stress plants suppress stomatal opening in order to conserve water (Copolovici et al., 2014; Nabi et al., 2019).

The role of nutrients in plant growth is explained of their functions in plant metabolism. The nutritional status can be affected by the adaptation capacity of plants, and also the plant tolerance abiotic/biotic stresses.

This study aimed to analyze the effect of drought stress on *Pisum sativum* L. var *Petit Provencal*, a commonly used garden pea variety in Romania, regarding photosynthetic parameters, and phenolic compounds.

MATERIALS AND METHODS

Plant material

Seeds of *Pisum sativum* L. (garden peas) were sowed in commercially available soil and nursed for one month prior to analyses. The experiment was carried out in the laboratories of the Institute for Research, Development and Innovation in Technical and Natural Sciences Aurel Vlaicu University in Arad. During the experiments, the plants have been between vegetative growth stages (V3) and reproductive growth stages (R5).

Determination of photosynthetic parameters

A gas exchange device GFS-3000 (Heinz Walz GmbH, Effeltrich, Germany) as described by Copolovici et al. (Copolovici et al., 2017) was used for the analysis of the photosynthetic parameters. The established measurements conditions were: PARtop = 1000 mmol. $m^2.s^{-1}$ for the light intensity, 25˚C enclosed leaf temperature, 70% chamber relative humidity and 400 ppm CO_2 concentration. Each measurement was performed by placing a 2-3 cm^2 plant offshoot in the 8 cm^2 analysis cuvette and left until the plant was stabilized. Assimilation rate and stomatal conductance were analysed.

Chemicals and reagents

Ethanol of 96% purity was purchased from Chemical Company (Romania), and used as solvent for the preparation of the phenolic extracts. Reference standards (phenolic compounds and vitamins) were purchased from Sigma-Aldrich (Germany). Other reagents and solvents used were of analytical purity, purchased from Sigma-Aldrich and Merck (Germany).

Induction of the drought stress

In the first day, all plants were watered to field capacity. Three plants were randomly chosen for drought treatment (no water provided until soil water capacity less than 50 %), while the remaining three were watered daily. After that period, the plants have been re-watered daily (recovery). The soil water capacity (SWC) has been determined using the following formula:

SWC (%) = (mass of moist soil (g) − mass of oven-dried soil (g))/(mass of oven-dried soil (g)) × 100

Phenolic extraction method

The plant material (pea seeds) were dried at 70ºC for 72 h using the drying oven (Model FD23, Binder, Germany). All extracts were prepared using 1:10 w/v pea flour in 60% ethanol solution using static maceration for 7 days in a refrigerator at +4ºC. All extracts were shaken from time to time and in the end, filtered using 0.45 μm PTFE membrane syringe filters.

UHPLC analysis of the phenolic extracts

HPLC analyses were performed using a UHPLC (Nexera X2, Shimadzu, Tokyo, Japan) with DAD (M30A, Shimadzu, Tokyo, Japan) and a Nucleosil reversed-phase column 100-3-C18 (4.0 mm i.d. x 125 mm column length, 3 μm particle size, Macherey-Nagel GmbH, Duren, Germany). The column temperature was set at 30ºC and the flow rate was maintained 1 mL/min. The elution program and solvents are as described by Moisă et al. (2018). The references standards used were: rutin, quercetin, kaempferol, catechin, pyrogallol, pyrocatechol, p-coumaric acid, caffeic acid, vanillic acid, syringic acid, ascorbic acid, and riboflavin.

RESULTS AND DISCUSSIONS

Photosynthetic parameters

The photosynthetic parameters for *Pisum sativum* L. control plants presented high values for stomatal conductance and a net assimilation rate. After the drought begin, the photosynthetic parameters decline linearly until very low values at 60 % relative soil water content (stomata closure).

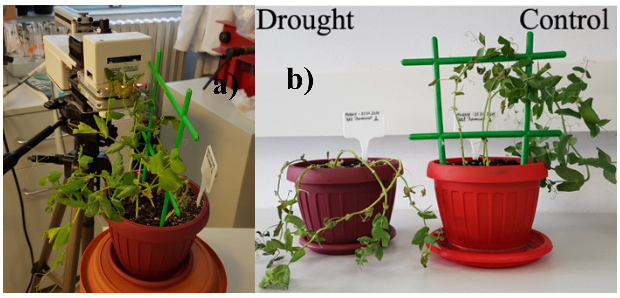

Figure 1. Monitoring photosynthetic parameters (a) and plants under stress (b) for *Pisum sativum* L. plants.

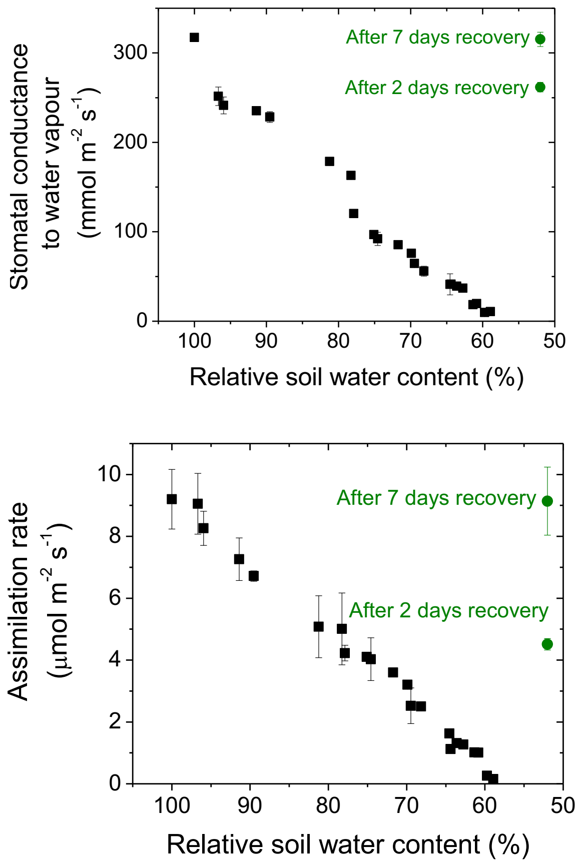

Figure 2. Stomatal conductance to water vapour and assimilation rate for *Pisum sativum* L. plants

Such behavior have been shown before for different plants species under drought stress (see (Sreeman et al., 2018) for review). After the plants have been watered, all parameters recovered at initial parameters (after 7 days). Such recovery could be due to interactions between nodule bacteria (rhizobia) which plays an important role in tolerance of *Pisum sativum* L. plants to drought (Belimov et al., 2019).

The influence of light to photosynthesis parameters

For each plant, periodic measurements were performed at different photosynthesis active radiance (0, 100, 200, 300, 400, 500, 600, 800, 1000, 1200 and 1500 µmol m^{-2} s^{-1}). The graphs obtained present the photosynthetic response of the plant to the light.

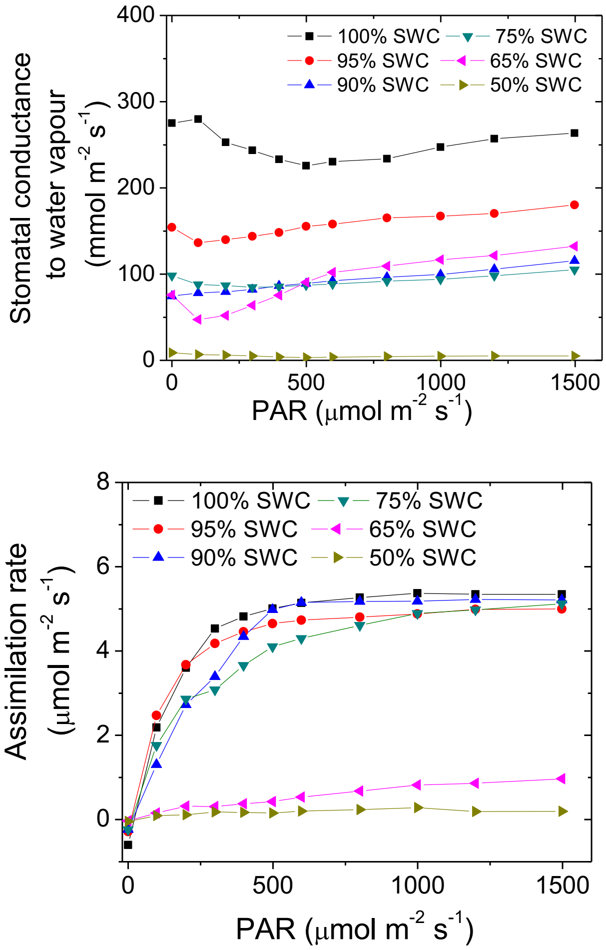

Figure 3. Light dependence of stomatal conductance to water vapour and assimilation rate for *Pisum sativum* L. plants.

The light dependence of those parameters has been shown a sharp decrease after 70 % relative soil water contents. Such behavior could be explained by a stomata closure in order to kept the water in the cells.

Phenolic composition

The extracts obtained from pea beans have significant amounts of phenolic compounds as it is presented in Table 1.

Table 1. HPLC analysis results (phenolics) of extracts from *Pisum sativum* L. seeds

Compound	Control mg/L	Drought mg/L
Riboflavin	205.86 ± 1.22	207.61 ± 7.16
Vanillic acid	2.15 ± 0.427	2.12 ± 1.053
Syringic acid	3.29 ± 0.213	2.42 ± 0.154
Catechin	0.014 ± 0.003	0.011 ± 0.002
Rutin	ND	ND
Quercetin,	ND	ND
Kaempferol	ND	ND
Pyrogallol	ND	ND
Pyrocatechol	ND	ND
Caffeic acid	ND	ND
p-Coumaric acid	ND	ND
Ascorbic acid	ND	ND

ND – not detected

The major phenolic compound in all investigated extracts was riboflavin with 207.61 mg/L, followed by syringic acid with 3.29 mg/L and vanillic acid with 2.15 mg/L, with no significant differences between control and drought plants.

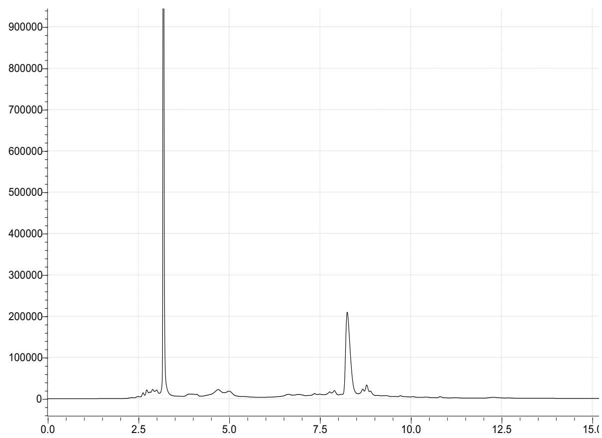

Figure 4. Example of phenolic compounds chromatogram of *Pisum sativum* L. extracts

CONCLUSIONS

Drought affects the photosynthetic parameters as assimilation rate and stomatal conductance to water vapors. The light dependence of those parameters has been shown a sharp decrease after 70 % relative soil water contents. Understanding how plants react to different environmental stressors is important for understanding climate change and its effects on food nutrition and safety.

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REFERENCES

Baigorri, H., Antolín, M.C., Sánchez-Díaz, M., 1999. Reproductive response of two morphologically different pea cultivars to drought. European Journal of Agronomy 10, 119-128.

Belimov, A.A., Zinovkina, N.Y., Safronova, V.I., Litvinsky, V.A., Nosikov, V.V., Zavalin, A.A., Tikhonovich, I.A., 2019. Rhizobial ACC deaminase contributes to efficient symbiosis with pea (*Pisum sativum* L.) under single and combined cadmium and water deficit stress. Environmental and Experimental Botany 167.

Cervenski, J., Danojević, D., Savic, A., 2017. Chemical composition of selected winter green pea (*Pisum sativum* L.) genotypes.

Copolovici, L., Kaennaste, A., Remmel, T., Niinemets, U., 2014. Volatile organic compound emissions from *Alnus glutinosa* under interacting drought and herbivory stresses. Environmental and Experimental Botany 100, 55-63.

Copolovici, L., Pag, A., Kännaste, A., Bodescu, A., Tomescu, D., Copolovici, D., Soran, M.-L., Niinemets, Ü., 2017. Disproportionate photosynthetic decline and inverse relationship between constitutive and induced volatile emissions upon feeding of *Quercus robur* leaves by large larvae of gypsy moth (*Lymantria dispar*). Environmental and Experimental Botany 138, 184-192.

Mejri, F., Ben Khoud, H., Njim, L., Baati, T., Selmi, S., Martins, A., Serralheiro, M.L.M., Rauter, A.P., Hosni, K., 2019. In vitro and in vivo biological properties of pea pods (*Pisum sativum* L.). Food Bioscience 32, 100482.

Moisă, C., Copolovici, L., Bungau, S., Pop, G., Imbrea, I., Lupitu, A.-I., Nemeth, S., Copolovici, D., 2018. Wastes resulting from aromatic plants distillation - bio-sources of antioxidants and phenolic compounds with biological active principles. Farmacia 66, 289-295.

Nabi, R.B.S., Tayade, R., Hussain, A., Kulkarni, K.P., Imran, Q.M., Mun, B.-G., Yun, B.-W., 2019. Nitric oxide regulates plant responses to drought, salinity, and heavy metal stress. Environmental and Experimental Botany 161, 120-133.

Nithiyanantham, S., Selvakumar, S., Siddhuraju, P., 2012. Total phenolic content and antioxidant activity of two different solvent extracts from raw and processed legumes, *Cicer arietinum* L. and *Pisum sativum* L. Journal of Food Composition and Analysis 27, 52-60.

Sreeman, S.M., Vijayaraghavareddy, P., Sreevathsa, R., Rajendrareddy, S., Arakesh, S., Bharti, P., Dharmappa, P., Soolanayakanahally, R., 2018. Introgression of physiological traits for a comprehensive improvement of drought adaptation in crop plants. Frontiers in Chemistry 6.

Tanase, C., Bujor, O.-C., Popa, V.I., 2019. Chapter 3 - Phenolic natural compounds and their influence on physiological processes in plants, in: Watson, R.R. (Ed.), Polyphenols in Plants (Second Edition). Academic Press, pp. 45-58.

Zilani, M.N.H., Sultana, T., Asabur Rahman, S.M., Anisuzzman, M., Islam, M.A., Shilpi, J.A., Hossain, M.G., 2017. Chemical composition and pharmacological activities of *Pisum sativum*. BMC complementary and alternative medicine 17, 171-171.