STUDY REGARDING INFLUENCE OF MICROWAVE IRRADIATION ON CHLOROPHYLL CONTENT AT SOME BARLEY (*HORDEUM VULGARE* L.) GENOTYPES

Iuliana Crețescu[1*], Rodica Căpriță[1], Giancarla Velicevici[1], A. Căpriță[1]

[1]*Banat's University of Agricultural Sciences and Veterinary Medicine, Timişoara, Romania*

Abstract

Chlorophyll is one of the major components of chloroplasts and represents a variable in assessing the physiological status and photosynthetic performance of plants. The purpose of the study was to evaluate the microwave irradiation effect on the chlorophyll content of Hordeum vulgare L. The chlorophyll content was determined at 14 days after the irradiation treatment, using the portable chlorophyll meter SPAD- 502 (Konica Minolta). We observed variations in the chlorophyll content when applying different power values of the radiations and different times of exposure. The chlorophyll content was lower for 720W irradiation power than for 400W, for all studied genotypes. The chlorophyll values ranged between 12.94 and 20.35 SPAD at irradiation time of 10s and between 17.26 and 20.87 SPAD for irradiation time of 20s. The chlorophyll content values in the control samples ranged between 19.94 and 22.26 SPAD. Experimental investigations of microwave irradiation on plant development may help to clarify the mechanisms of energy exchange in molecules and thus stimulation of plant development.

Key words: microwave irradiation, barley genotypes, chlorophyll content

INTRODUCTION

Cereals represent an integral part in global agriculture and diet. Barley (*Hordeum vulgare L.*) is one of the most important crop species in the world and is the subject to considerable genetic studies. It is a diploid (2n=2x=14), largely self-fertilizing species with a large genome [2]. Together with genetics and biochemistry, biophysics plays a key role in the improvement of agricultural production and production of new genotypes.

The aim of our investigation was to determine the effect of microwave treatment on the chlorophyll content of four Romanian barley genotypes.

The microwave radiation is electromagnetic radiation with frequencies between 0.3GHz and 300GHz. Most investigated is the radiation 2450 MHz because it is absorbed by water molecules, present in all live cells. Microwave energy penetrates a food or feed material and due dipolar rotation of polar solvents. The dipolar rotation is caused by variations of the electrical and magnetic fields in the organic components. Water is the main source for microwave interactions due to its dipolar nature.

Chlorophyll is one of the major components of chloroplasts and the chlorophyll content is positive correlated with photosynthesis rate. Chemical structure discovering of chloro-phyll in 1915 by Richard Willstätter, taken Nobel prize for him. It is a mixture of two compounds: chlorophyll a and chlorophyll b. Chlorophyll *a* is common for all photosynthetic eucariot organisms by which the plants, algae, protozoa and some bacteria convert solar energy in chemical energy.

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

Relative changes in photosynthetic pigments could be used to assess water deficit effects in the plants [8].

Larcher (1995) affirmed that variations in chlorophyll content can be caused by water stress but also by phenological status of the plant, atmospheric pollution, nutrient deficiency, toxicity, plant disease, and radiation stress. Some plants, therefore, have developed a strategy to protect the chloroplasts which are vital components. As such, a

*Corresponding author: iulianacretescu@usab-tm.ro
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decrease of water content does not always result in a decrease in chlorophyll content.

The estimation of chlorophyll content is an alternative to the estimation of vegetation water content and thus an alternative the estimation of vegetation status. A number of studies are based on the relation between chlorophyll and water content. [4, 9, 10]

MATERIAL AND METHOD

The biologic material used in that study was represented by a collection of 4 genotypes of Romanian winter barley - Maresal, Sistem, Dana and Cardinal, obtained from the Fundulea Agriculture Research Station.

A magnetron MWG20H with a frequency of radiation of 2450 MHz and maximum output power of 800W, according to supplier's data, has been used as microwave source. The seeds were initially treated with 1.5% sodium hypochlorite for 15 min. The residual chlorine was eliminated by thorough washing of the seeds with distilled water. The seeds have been exposed to the microwave radiation for 0s (control-M), 10s, 20s, with two modifications of output powers of the magnetron – 400W and 720W, corresponding intensities – 20kW/m^3 and 36kW/m^3 respectively, have been applied. The seeds were then germinated on filter paper in Petri dishes, in growth chamber. The experiments have been performed under laboratory conditions. The natural light cycle was 9 h of light/14h of darkness and the daily temperature was 21 ± 2ºC, night temperature 15 ± 2ºC. The chlorophyll content of leaves was determined by portable chlorophyll meter SPAD - 502 (Konica Minolta) at 14 days after sowing, for every variant. Experimental data had been processed by statistical methods [3] and Principal Component Analysis method.

RESULTS AND DISCUSSIONS

Vegetation status is an indicator of the degree of stress experienced by plants in their environment [6]. Vegetation stress can be defined as any disturbance that adversely influences growth [5]. This stress can be due to many factors, one of which is a lack of water that restricts transpiration, inducing closure of stomata and resulting in less water evaporating from the leaf surface.

Chlorophyll is one of strong antioxidant substances known until present. So, high chlorophyll content lead on significant decrease of oxidative effects induced by factors stress.

The reduction of chlorophyll content was considered a typical symptom of oxidative stress that could be the result of pigments photobleaching and chlorophyll degradation [1].

The level of chlorophyll content for all genotypes and variants experimental can be observed in Table 1. The data in Table 2 show the effect of barley varieties on average chlorophyll content (SPAD). The effect of the time of microwave exposure and microwave power on the average chlorophyll content (SPAD) of barley seedlings can be observed in Tables 3 and 4.

Table 1 The effect of microwave irradiation on the average chlorophyll content (SPAD) of barley seedlings

Genotype	Variant	14 days			
		$\overline{X} \pm s_{\overline{X}}$		CV (%)	
		Time of exposure (s)		Time of exposure (s)	
		10	20	10	20
Sistem	0W	21.33±1.96	21.33±1.96	29.02	29.02
	400W	22.41±2.22	20.03±1.24	31.36	19.57
	720W	20.35±1.62	17.6±1.64	25.25	29.54
Maresal	0W	22.26±1.17	22.26±1.17	16.71	16.71
	400W	18.77±0.95	21.49±1.15	16.08	16.98
	720W	18.56±1.39	20.87±1.30	23.70	19.70
Dana	0W	21.03±0.97	21.03±0.97	14.64	14.64
	400W	19.3±1.29	17.9±1.25	21.13	22.17
	720W	15.65±1.74	17.26±1.28	35.2	23.46
Cardinal	0W	19.94±2.38	19.94±2.38	37.81	37.81
	400W	19.80±0.79	22.04±0.93	12.62	13.43
	720W	12.94±1.07	20.71±1.52	26.19	23.27

Table 2 The effect of barley varieties on average chlorophyll content (SPAD)

No.	Varieties	SPAD		Rel. val (%)	Significance
1	Maresal - Sistem	20.39	20.34	100.24	0.05
2	Dana - Sistem	18.22	20.34	89.57	-2.12^{000}
3	Cardinal - Sistem	19.08	20.34	93.80	-1.26^{000}
4	Dana - Maresal	18.22	20.39	89.35	-2.17^{000}
5	Cardinal - Maresal	19.08	20.39	93.57	-1.31^{000}
6	Cardinal - Dana	19.02	18.22	104.39	0.8^{**}

LSD5%=0.30; LSD 1%=0.55; LSD 0.1%=1.23

Table 3 The effect of the time of microwave exposure on the average chlorophyll content (SPAD) of barley seedlings

Time of exposure	SPAD		Rel. val. (%)	Difference/ Significance
10sec - 0sec	18.47	21.14	87.36	-2.67^{000}
20sec - 0sec	19.73	21.14	93.33	-1.41^{00}
20sec - 10sec	19.73	18.47	106.82	1.26^{**}

LSD5%=0.33; LSD 1%=0.78; LSD 0.1%=2.49

Table 4 The effect of the power of microwave on the average chlorophyll content (SPAD) of barley seedlings

Time of exposure	SPAD		Rel. val. (%)	Difference/ Significance
400W - 0W	20.21	21.14	95.6	-0.93^{0}
720W - 0W	17.99	21.14	85.09	-3.15^{0}
720W - 400W	17.99	20.21	89.01	-2.22^{0}

LSD5%=0.75; LSD 1%=3.77; LSD 0.1%=37.72

The quantity of chlorophyll of cultivars taken in study, registered values between 20.71 SPAD at Cardinal genotype for 720 W power of microwave irradiation at 20 s time of exposure and 12.94 SPAD at Cardinal genotype for 400W power of microwave irradiation at 10 s time of exposure (table 1). Regarding the unilateral effect of power irradiation and time of exposure on chlorophyll content at barley seedlings, that presented values between 12.94 SPAD and 20.35 SPAD on variant 720W and between 18.77 SPAD and 22.41 SPAD on variant 720W for 10 s time of exposure and between 17.26 SPAD and 20.87 SPAD on variant 720W for 10s time of exposure and between 17.9 and 22.04 SPAD on variant 400W for 20s time of exposure. The genotypic variation in microwave treatment is observed for chlorophyll content, as the parameter involved in photosynthesis.

The treatments applying had determined decrease of chlorophyll content proportional with increased of the power of exposure. According to presented data in table 3 it could observe as time of exposure had influence on chlorophyll accumulation to genotypes studied. For the coefficient of variation it was observed that for most genotypes chlorophyll content had a medium variability. The highest results for chlorophyll content have been obtained when barley seeds were treated with the microwave output power of 400 W.

Correct application of physical methods of stimulation requires experimental investigation and establishment of convenient regimes, which for all the studied cases strongly depends on plant characteristics, intensity of microwave and exposure time.

Determination of the chlorophyll content may be applying of an indirect test method the specific electroconductivity, based on the possible hypothesis about the absorption of the microwave radiation energy by the hydrogen or magnesium atom electrons in the chlorophyll molecule an indirect test method to drought tolerance. The energy absorbed is redistributed and it causes changes in the chlorophyll molecule. By increasing the radiation power used for the treatment of the samples, the amount of free ions in the extract decreases and hence its electroconductivity, too. A reduced concentration of photosynthetic pigments could determine a direct reduction of photosynthetic potential and implicitly of production.

Genotypes which registered higher chlorophyll content had resulted to be more tolerant to microwave action for high power of microwave. Photosynthetic efficiency of crops may be associated with resistance to stress by measuring the fluorescence of chlorophyll a [11].

This finding suggests the existence of a certain relationship between the genetic factors defining the tolerance towards the high power of microwave of the genotype and its resistance to dehydration.

CONCLUSIONS

1. It can be noticed that the highest results chlorophyll content have been obtained after barley seeds were treated with the microwave output power of 400 W for Sistem, Maresal and Cardinal genotypes at 20s time of exposure. Photosynthetic pigments were also positively affected by microwave exposure.

2. Experimental investigations of the microwave influence on plant development may help to clarify the mechanisms of energy exchange and thus stimulation of plant development.

3. Chlorophyll fluorescence techniques can be used to determine photosynthetic rates of cereals leaves much more rapidly than is possible with conventional gas exchange methods. SPAD meter could be used to provide a rapid estimate of leaf absorbance in the field.

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