BIOCHEMICAL EFFECTS INDUCED BY UV TREATMENT ON 5 ROMANIAN *PHASEOLUS VULGARIS L.* CULTIVARS, GROWN IN FIELD

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Key words: UV-B, bean, cultivar, sample

Abstract: Our study is focused on the influence of the UV-B irradiation, at the level of hidric balance, methabolic activity, and in the content of minerals, polyphenols, pigments, nucleic acids, proteins, of five romanian cultivars: *Diva, Star, Vera, Ami, Avans* of ***Phaseolus vulgaris,*** sawn after germination in enreached UV-B environment in natural field condition**.**

INTRODUCTION

Due to the increased level of UV-B radiation, λ=290-320nm, (reaching Earth surface as a consequence of stratospheric ozone layer depletion), there might appear in plants as fixed organisms, either stress or aclimatization responses, consisting in molecular, fiziological or morpho-anatomical changes.Our study intends to determine the effects of UV-B irradiation on five bean *Phaseolus vulgaris* cultivars, at biochemical level.

MATERIAL AND METHODS

Biological material: *Phaseolus vulgaris* seeds of 5 romanian cultivars

Mutagenic agent: UV-B radiations. Light source: TL 40 W/12 (λ_{max} 310nm, fluence 19,6 Wm^{-2} 30cm irradiation high).

Working steps:

1. 100 seeds from every variant were germinated for 48h, in Petri dishes without cover, on filter paper imbibated with distilled water, under UV-B lamp.
2. Irradiated and not irradiated (control) seeds were sawn in experimental field from Podu Iloaiei, Iasi, according to the agro-technical standards specific for Moldavian plateau.
3. After reaching biological maturity, at the end of vegetative phase, plants were biochemical investigated.

Water and dried substance content analysis was made by 2g sample drying at 105^0C for 4 hours, in open vials in an air flow, followed by placing quickly covered vials in an anhydrous $CaCl_2$ exicator, and weight after cooling down to room temperature (in about 1 hour).

$U\% = \frac{G - G1}{G} \cdot 100$, where: U=humidity; G=sample weight before drying (g); G1; sample weight after drying (g). 100-U represents dried material at 105^0C, expressed in procents.

Ash percentage determination: 2g from each sample were placed in previously calcined crucible and treated with 3ml alcohol 90 $^{c.}$. Alcohol was burn till obtaining a loose white or grey ash, or till any weight loss can be measured anymore (few hours).

Ash percentage is expressed comparing to air dried substance.

$c(\%) = \frac{m1}{m2} \cdot 100$, where: c%= ash percentage; m1= ash weight (g); m2= sample weight (g).

Determination of **soluble substances content** was done by extraction for 23h, from 5g dried and powdered vegetal material, using 100ml solvent. After filtration, 20ml filtrate was evaporated on water bath and dried at 105ºC to constant weight, which was reported to 100g vegetal material.

Determination of **flavonoids content** (expressed in rutozid) was done by extraction for 30 minutes, from 2g sample, with 40 ml alcohol 50% by warming on water bath. After cooling and filtration, 50% alcohol was added to filtrate, up to 50ml (A solution). For each variant, 2,5ml from A solution, 5ml sodium acetate (100g/l) and 3ml aluminium chloride (25g/l) were mixed after each reactive addition, and filled up to 25ml with alcohol 50%. 15 minutes after last reactive, samples and control (same with sample but without aluminium chloride) absorbance was read at 430nm.

To calculate total flavonoids content it is used specific extinction of 1% standard rutozid solution:

% flavone = $\frac{A \cdot f \cdot n}{a \cdot v} \cdot 100$, where A= sample absorbance; f= solution factor (0,975x 10^{-3}); n= sample volume; a= sample weight; v= A solution volume for colorimetric reaction (ml).

To determinate **total poliphenols content**, 2g of each sample are used for extraction 30 minutes, with 40 ml alcohol 50% by warming to boil on water bath (A solution). In a vial 1ml A solution is mixed with 1ml Folin reactive (B solution). 0,2ml from B solution is transferred to another vial and mixed with sodium carbonate. Samples extinction is read at 660nm. Standard solution is cafeic acid.

% poliphenols= $\frac{A \cdot f \cdot v}{a \cdot n} \cdot 100$, where A=absorbance; f= solution factor (0,138x 10^{-3}); v= sample volume; a= sample weight (g), n= ½ B solution volume for colorimetric reaction.

Determination of **total acidity**: 0,5g from each sample were mixed with 40ml distilled water. After filtration, glass and filter were washed 3 times with 5ml distilled water each time. Filtrate and washing steps water were mixed and titrated with NaOH 0,1N in presence of phenolphtaleine 1%, till colur turns to redish-yellow. Total acidity is expressed in malic acid:

g% malic acid= $\frac{V \cdot 0{,}0067 \cdot f}{a} \cdot 100$ where: V= NaOH 0,1N volume used to titrate (ml); f= NaOH 0,1N solution factor; a= sample weight used for determination.

RESULTS AND DISCUSSIONS

Water and dried substance content as shown in Tab. 1, Fig. 1 decrease in the case of irradiated samples for all of the cultivars, more evidently for *Diva* and *Avans* cultivar.

Tab. 1, Water content before and after irradiation for 5 *Phaseolus vulgaris* cultivars

Humidity (%)			
Nr.	**Cultivar**	**Controle**	**Irradiated sample**
1	DIVA	7,74	7,17
2	STAR	8,09	8,07
3	VERA	8,39	8,2
4	AMI	8,10	7,83
5	AVANS	7,81	7,35

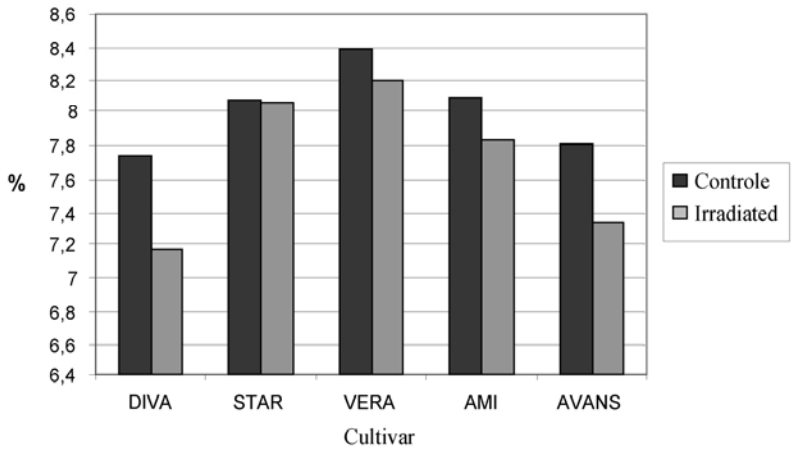

Fig.1, Water content before and after irradiation for 5 *Phaseolus vulgaris* cultivars

Ash percentage increase after irradiation, for all cultivars, more evidently for *Star* (Tab. 2, Fig. 2). Less increase were observed for *Diva, Vera*, *Ami, Avans.*

Tab. 2. Ahs content before and after irradiation for 5 *Phaseolus vulgaris* cultivars

Ash (g%) reported to dry substance.			
Nr.	**Cultivars**	**Controle**	**Irradiated sample**
1	DIVA	6,69	7,37
2	STAR	4,78	10,25
3	VERA	4,79	5,6
4	AMI	4,8	5,6
5	AVANS	5,2	5,3

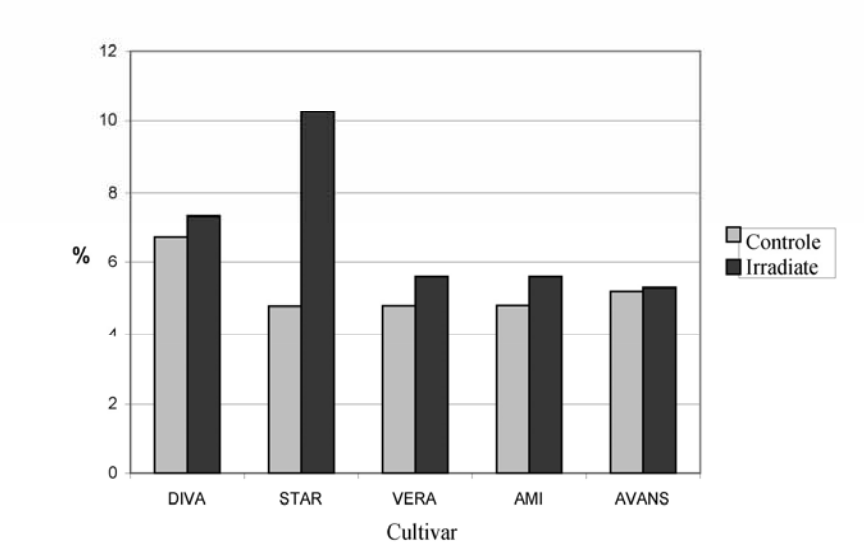

Fig. 2, Ahs content before and after irradiation for 5 *Phaseolus vulgaris* cultivars

Determination of soluble substances content shows an increase for the irradiated samples, more evidently for *Diva*, *Vera* and *Avans* cultivars, by accumulation of substances like flavonoids, polyphenols, aminoacids, (Tab. 3, Fig. 3).

Tab. 3. Soluble substances content before and after irradiation for 5 *Phaseolus vulgaris* cultivars

Ash (g%) reported to dry substance.			
Nr.	**Cultivars**	**Controle**	**Irradiated sample**
1	DIVA	15,24	19,32
2	STAR	12,32	14,46
3	VERA	13,1	19,84
4	AMI	14,06	15,22
5	AVANS	14,44	18,5

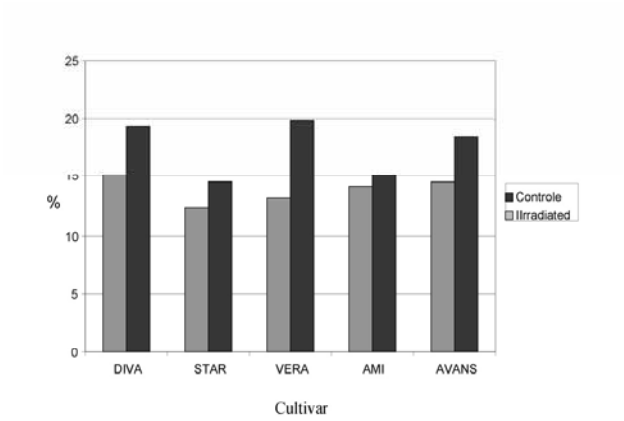

Fig.3 Soluble substances content before and after irradiation for 5 *Phaseolus vulgaris* cultivars

Flavonoids content representing a protective mechanism as screen pigment, increase after UV irradiation for all cultivars, more evidently for *Ami.* In the case of *Avans* cultivar the increase rate is low, suggesting that this cultivar is the most sensitive at UV irradiation (Tab. 4, Fig. 4).

Tab. 4. Flavonoids content before and after irradiation for 5 *Phaseolus vulgaris* cultivars

Flavonoids (g%) reported to dry substance			
Nr.	**Cultivars**	**Controle**	**Irradiated sample**
1	DIVA	0,04	0,094
2	STAR	0,027	0,064
3	VERA	0,029	0,054
4	AMI	0,047	0,175
5	AVANS	0,043	0,047

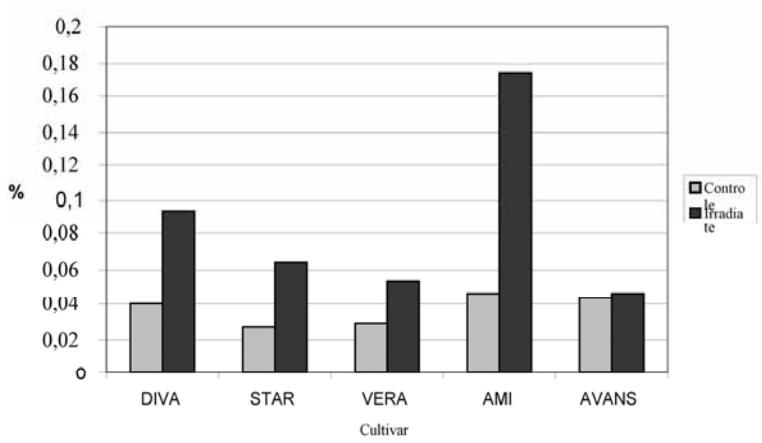

Fig. 4. Flavonoids content before and after irradiation for 5 *Phaseolus vulgaris* cultivars

After determination of total poliphenols content, it can be conclude that it increase for all cultivars after irradiation, but with a width range of variation (up to 0,230g% for *Diva* and 0,104g% for *Vera*) (Tab. 5., Fig. 5). The poliphenols content increase can be considered as a protective mechanism against possible UV induced damage. After

Caldwell *et al.*, 1983; Beggs *et al*.,1986; Beggs & Wellmann, 1994, flavonoids and poliphenols content increase after UV exposure in the leaves upper epidermal layer, acting as a protective filter against radiations.

Tab. 5. Poliphenols content before and after irradiation for 5 *Phaseolus vulgaris* cultivars

Poliphenols (g%) reported to dry substance			
Nr.	**Cultivars**	**Controle**	**Irradiated sample**
1	DIVA	0,083	0,230
2	STAR	0,117	0,131
3	VERA	0,097	0,104
4	AMI	0,155	0,214
5	AVANS	0,097	0,149

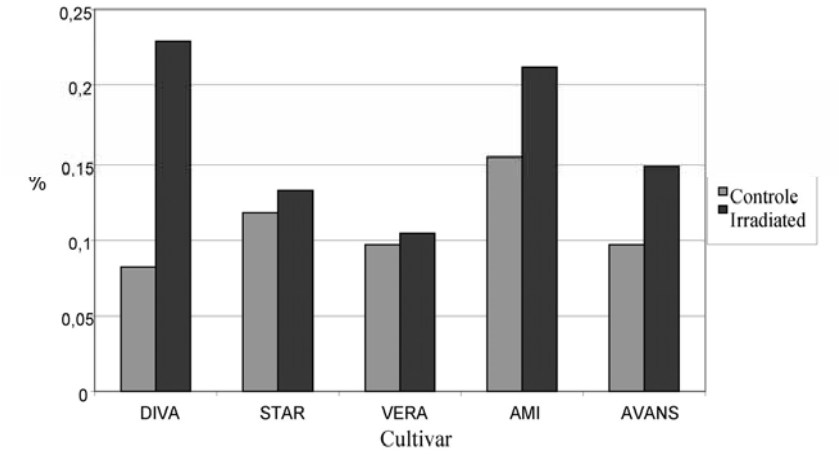

Fig. 5. Poliphenols content before and after irradiation for 5 *Phaseolus vulgaris* cultivars

Total acidity increase after irradiation for each experimental variant, more evident for *Vera* cultivar, followed by *Avans,* and than *Ami Star* and *Diva*, as shown in Tab. 6, Fig. 6.

Tab. 6. Total acidity content before and after irradiation for 5 *Phaseolus vulgaris* cultivars

Total acidity (%)reported to dry substance			
Nr.	**Cultivars**	**Controle**	**Irradiated sample**
1	DIVA	1,30	1,346
2	STAR	0,783	0,837
3	VERA	0,764	1,154
4	AMI	0,75	0,867
5	AVANS	1,193	1,467

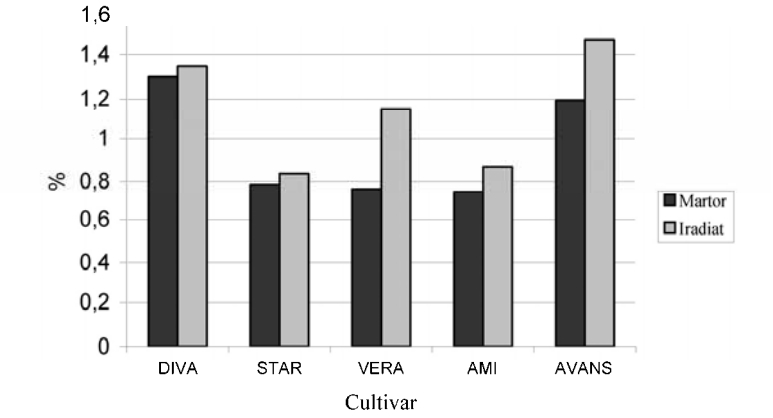

Fig. 6. Total acidity content before and after irradiation for 5 *Phaseolus vulgaris* cultivars

CONCLUSIONS

The UV irradiation induce a change in water balance, expressed by decrease of humidity.
The dry and soluble substance content increase as response to UV irradiation stress, as a consequence of general metabolism stimulation.
The flavonoids and polyphenols content, representing screening pigments, increase as respons to UV stress.
The UV irradiation induce the increase of total acidity for all the investigated cultivars.

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