## IZOFLAVONOID SYNTHETIS AT BEAN *PHASEOLUS VULGARIS* L., AS RESPONSE TO UV IRRADIATION

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

**Abstract**: Our study is focused on the influence of the UV-B irradiation, at the level of izoflavonoids (3-arilocumarine and cumestrole) synthetis capacity of bean, *Saxa* cultivar, beeing known that this izoflavonoids are synthetised in leaves due to UV-B irradiation, being used as a cuantificabile marker for induction of damages at DNA level, consisting in pirimidinic dimers.

#### INTRODUCTION

The scientific world researches, focused in the last decades, because of stratospheric ozone lay depletion, on the effects of UV irradiations (particulary UV-B), on organisms survival, on the induction of changes at the level of biochemical and genetic processes, and on the variability of characters.

For *Leguminosae*, DNA can act as a photoreceptor which induce izoflavonoids synthetis, even constitutive or as stress response. At *Phaseolus vulgaris*, izoflavonoids as cumestrole and 3-arilocumarine are synthetised in leaves due to UV-B irradiation, being used as a cuantificabile marker for induction of damages at DNA level, consisting in pirimidinic dimers, all this explaining (next to the importance of seeds and legumes in humans nutrition because of the increased level of high quality proteins, and the high energetically level, and due to the importance in soil amelioration), the choice of *Phaseolus vulgaris* as biological material for investigations.

In the present paper, the aim was to check and to present the results of investigations concerning the izoflavonoids synthetis capacity of bean, *Saxa* cultivar, in other experimental conditions than those described in literature.

#### MATERIAL AND METHODS

It is known that izoflavonoids needs time to acumulate in irradiated leaves. There were 2 experimental variants:24h, respective 48h accumulation time. For both variants it was tested also fotoreparation of dimers, by subsequent UV-A irradiation.

By migrating on chromatography silicagel plate, izoflavonoids were separated in the case of each experimental variant in 2 spots: a lower one which represents 3-arilocumarine, and a higher one which represents cumestrole. The dosage of 3-arilocumarine was made by spectrophotometer at  $\lambda$  342nm, and of cumestrole at  $\lambda$  =344nm.

For both experimental variants, it was proved UV-B izoflavonoids synthesis induction (by comparision of quantity with the quantity measured in non irradiated controle leaf), and fotoreparation (when part of thymine dimers were removed).

Comparing results obtained for both types of izoflavonoids, for both: 24h or 48h accumulation period, it can be observed that in 48h more izoflavonoids were accumulated than in 24h. It can be concluded that izoflavonoids are synthetised in time, and the quantity depends directly of dimers number formed in DNA. Even if izoflavonoids synthesis is not a specific response of UV stress, it can be proved by their synthesis DNA damage, estimating by correlation with their measured quantity the level of damage.

Izoflavonoids were extracted for each experimental variant, from 10 rondels of 0,785cm<sup>2</sup> each. By migrating on chromatography silicagel plate, izoflavonoids were separated (for both 24h, respective 48h accumulation time following 30 minutes UV WG360 or Q irradiation of lower epidermis of 12 days old primary leafs), in 2 spots: a lower one which represents 3-arilocumarine, and a higher one which represents cumestrole. The dosage of 3-arilocumarine was made by spectrophotometer at  $\lambda$  342nm, and of cumestrole at  $\lambda$  =344nm. For both variants it was tested also fotoreparation of dimers, by subsequent UV-A irradiation (for 30 minutes).

#### **RESULTS AND DISCUSSIONS**

In 48h more izoflavonoids were accumulated than in 24h.

Tab.1. 3-arilocumarine synthetised in *Phaseolus vulgaris* primary leaves, accumulation time 24h

| Irradiation Variant |              |  |  |
|---------------------|--------------|--|--|
| Q / WG 360          | Q / Q + UV-A |  |  |

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| Q      | WG 360 | Q     | Q + UV-A |
|--------|--------|-------|----------|
| 0,0796 | 0,0386 | 0,195 | 0,192    |

Tab.2. Cumestrole synthetised in *Phaseolus vulgaris* primary leaves, accumulation time

24h

|   | Q / Q        | ) + UV-A |
|---|--------------|----------|
|   | Q / Q + UV-A |          |
| ) | Q            | Q + UV-A |
|   | 0,0309       | 0,0195   |
|   |              |          |

Tab.3. 3-arilocumarine synthetised in *Phaseolus vulgaris* primary leaves, accumulation time 48h

| Irradiation Variant |            |        |              |  |  |
|---------------------|------------|--------|--------------|--|--|
| Q / Y               | Q / WG 360 |        | Q / Q + UV-A |  |  |
| Q                   | WG 360     | Q      | Q + UV-A     |  |  |
| 0,0261              | 0,0121     | 0,1711 | 0,0583       |  |  |

Tab.4. Cumestrole synthetised in *Phaseolus vulgaris* primary leaves, accumulation time 48h

| Irradiation Variant |            |        |          |  |  |
|---------------------|------------|--------|----------|--|--|
| Q / V               | Q / WG 360 |        | UV-A     |  |  |
| Q                   | WG 360     | Q      | Q + UV-A |  |  |
| 0,0286              | 0,0135     | 0,0853 | 0,0348   |  |  |

It can be concluded that izoflavonoids are synthetised in time, and the quantity depends directly of dimers number formed in DNA. Even if izoflavonoids synthesis is not a specific response of UV stress, it can be proved by their synthesis DNA damage, estimating by correlation with their measured quantity the level of damage.

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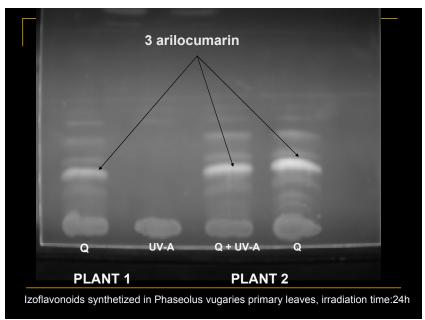


Fig. 1. 3 arilocumarin synthesis in Phaseolus vulgaris

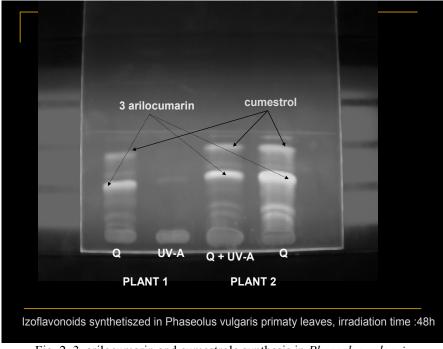


Fig. 2. 3 arilocumarin and cumestrole synthesis in Phaseolus vulgaris

#### CONCLUSIONS

In 48h more izoflavonoids were accumulated than in 24h.

Izoflavonoids are synthetised in time.

The izoflavonoids quantity depends directly of dimers number formed in DNA.

Even if izoflavonoids synthesis is not a specific response of UV stress, it can be proved by their synthesis DNA damage, estimating by correlation with their measured quantity the level of damage (they can be used as cuantificabile marker).

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