

## **IN VIVO AND IN VITRO FLAVONOID PRODUCTION IN *LOTUS TENUIS* WALDST. ET KIT.**

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A quali- and semiquantitative research of the flavonoids present in *Lotus tenuis* was performed in order to characterize the species and to determine the chemosystemic value of those compounds.

Firstly, the flavonoids present in the shoots of plants in vegetative stage were isolated; free kaempferol (K) and kaempferol-3-O-glucosyl-7-O-rhamnosyde (K-G-R) were identified [1].

Then, it was intended to determine if the relative concentration of those compounds varied during the whole life cycle of the plant. Therefore different samples were collected during the vegetative, floral bud, flowering and fruiting stages.

It was proved the existence of a rhythm of production for the kaempferol-3-O-glucosyde (K-G). The latter only appearing during the reproductive stage [2] (Fig.1).

In order to understand the biosynthetic pathways and also the function of the flavonoids in *L. tenuis*, vegetal material from *in vitro* culture was analyzed (see Materials and Methods).

The presence of flavonoids in the *calli* could not be detected, while in the plants regenerated from the *calli* the production of these compounds showed the same pattern that in the plants grew in the field.

The obtained results suggest that:

1. Flavonoids in *L. tenuis* would not function as phytoalexins; that is to say that they would not be defense agents against possible environmental aggression, as they are produced not only under aseptic conditions but also under natural culture conditions.

2. Flavonoid production would be associated to tissular differentiation mechanisms. This fact results particularly notorious in the shoots of the plants regenerated from the *calli* when compared to the shoots of the plants grew in the field.

3. A biosynthetic pathway for the production of K-G would be activated at the beginning of the reproductive stage. This fact is reaffirmed by the lack of this compound during the vegetative stage.

4. As regards the chemosystematic interest of the flavonoids it could be considered that they are of great help to solve problems of classification within *Lotus genus* [3,4]. It is important to take into account the phenological stage of the specimen to be studied in order not to misunderstand the obtained results [5]. Among the flavonoid compounds isolated in *L. tenuis* the free K and the K-G-R would be the most appropriate ones for these studies.

## EXPERIMENTAL

### 1. Flavonoids analysis

Plants of *L. tenuis* cultivar Chajá growing in the field were collected from the Introduction Garden at Chascomús (Province of Buenos Aires).

Plant material was air dried at room temperature and then separated into leaves, stems, flowers and fruits. 5 g of each fraction were ground and then extracted with boiling water during 20 minutes. The aqueous solution was subjected to reduced pressure and the residue redissolved in methanol.

Flavonoid compounds were isolated, purified and identified according to standard procedures described in a previous paper [1].

### 2. *In vitro* culture

Seeds of *L. tenuis* cv. Chajá were scarified in concentrated sulphuric acid and germinated on Murashige and Skoog (MS) agar solidified medium [6] with micronutrients at half strength. 30 g/l of sucrose and 8 g/l of agar were added to the culture medium.

The cotyledons from aseptically grown seedlings were used as explants for the regeneration assay and were incubated on MS agar solidified medium with the addition of an auxin-cytokinin relation 1:10 [7,83]. The culture conditions were:  $24 \pm 1^\circ\text{C}$  under fluorescent light (1,8 W/m; 16 h photoperiod).

The shoot formal ion was achieved after 40-45 days of culture, from previous *calli* and as a result of the dedifferentiation of the original explants.

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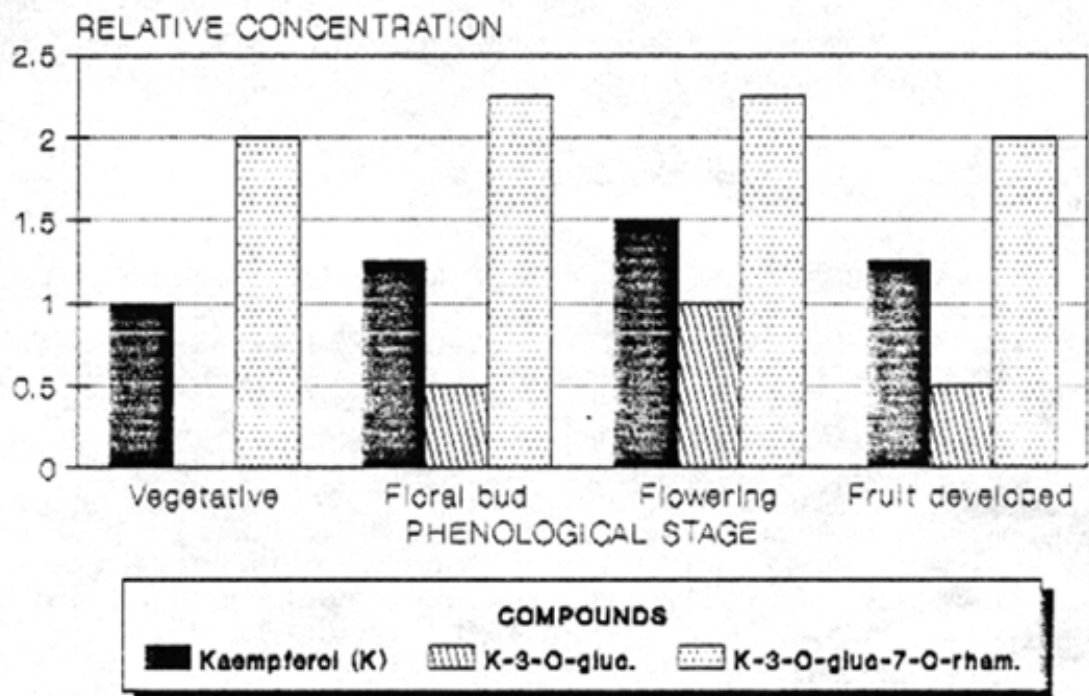


Fig. 1: Relative concentration of each detected compound during plant development.