

## A comparative study of the biochemical compounds found in three species of *Vigna* (*Vigna angularis* L., *Vigna mungo* L. and *Vigna radiata* L.) under salt stress conditions

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**ABSTRACT:** Saline soils are an unfavourable environment for the growth of most legumes. The aim of this work is to compare the biochemical behaviour of three species of bean (*Vigna radiata* L., *Vigna mungo* L., and *Vigna angularis* L.) under salt stress conditions. In order to assess this behaviour, we analysed polyphenols and flavonoids in the roots and leaves. The results reveal variability in the accumulation of polyphenols and flavonoids, depending on the organ of the plant, the species, and the intensity of the saline treatment.

**KEYWORDS:** NaCl, bean, polyphenol, flavonoid.

### 1 INTRODUCTION

Soil salinity is one of the main sources of abiotic stress, limiting the growth of cultivated plants (1). The salinisation of soils and irrigation water recorded in arid and semi-arid ecosystems, particularly in the Mediterranean basin, is one of the factors that limits plant productivity and crop yield (2), (3),(4). Almost 10% of land is affected by salt, and 10 million hectares of farm land are lost every year (5). The effects of salinity, in addition to the toxic effect caused by Na<sup>+</sup> and Cl<sup>-</sup> ions, are very similar to those of drought. As a result, plants adapt, reducing their water loss in order to maintain their vital functions (6). Salinity leads to water deficiency in plants, which is caused by osmotic stress, and is sometimes combined, with biochemical disturbances induced by the influx of sodium ions (7). Identifying the varieties and genotypes that are salt-tolerant and capable of minimising the depressive effects of salinity on yield would almost certainly make it possible to improve crop yield in areas affected by salinity (8).

We focused on the *Vigna* species and explored the effect of salinity on its biochemical behaviour. This plant was chosen for its ecological, economic and nutritive qualities. The study looks at three species (*Vigna radiata* L., *Vigna mungo* L., and *Vigna angularis* L.) and was carried out in saline conditions in a controlled greenhouse environment. To evaluate the responses of the plants, we analysed the variations in polyphenols and flavonoids at the level of the leaves and roots.

### 2 MATERIALS AND METHODS

#### 2.1 CROP MANAGEMENT

The experiments were carried out in a controlled greenhouse at the Laboratory of Plant Physiology. The seeds of three species were sown in cells filled with moist compost. These cells were placed in a greenhouse until the five-to-six leaf stage. The seedlings were then planted out in pots filled with sand and compost (2V/V).

#### 2.2 APPLYING THE STRESS

From the 21<sup>st</sup> day, the plants were divided into 3 batches of plants (controls, a first batch at 100 mM and a second batch at 200mM of NaCl. l<sup>-1</sup> of nutrient solution). Each batch was repeated 6 times for each species and under each treatment.

After one week of stress, samples of plants were taken and the leaves and roots were separated before being weighed. The analyses were then carried out.

### 2.3 ASSAY OF POLYPHENOLS

Polyphenols were extracted using the method described by (9). The polyphenol content is expressed in  $\text{mg.g}^{-1}$  DW after reading the optical density at 737 nm using a UV-VISIBLE spectrophotometer.

### 2.4 ASSAY OF FLAVONOIDS

Flavonoids were extracted using the method described by (10). Content is expressed in  $\mu\text{g.g}^{-1}$  DW after reading the optical density at 510 nm using a UV-VISIBLE spectrophotometer.

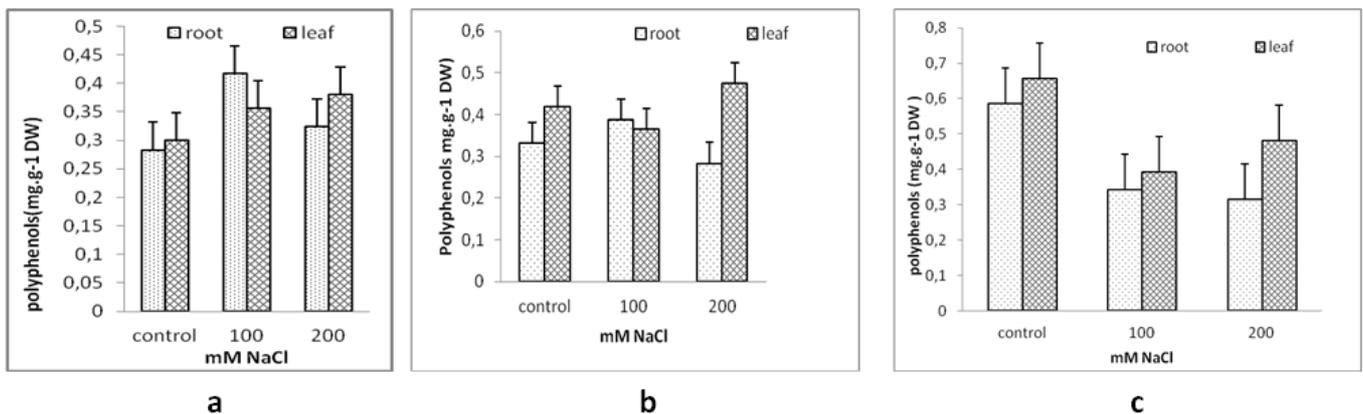
### 2.5 STATISTICAL ANALYSES

The results obtained underwent analysis of variance, using the software SPSS Statistics version 2.0. The results are represented as an average  $\pm$  standard deviation of the type  $p = 0.05$ .

## 3 RESULTS

### 3.1 EFFECT OF SALINITY ON THE POLYPHENOL CONTENT OF LEAVES AND ROOTS

The accumulation of phenolic compounds in *Vigna angularis* L. (Fig. 1a) evolved in the root-leaf direction in the control plants. In the roots, the polyphenol values recorded were  $0.43 \text{ mg.g}^{-1}$  DW with the treatment at 100 mM of NaCl, whereas when the plants were exposed to 200 mM, the phenolic compound content dropped to  $0.31 \text{ mg.g}^{-1}$  DW. On the contrary, in the leaves, the polyphenols accumulated more when the NaCl concentration of the milieu increased, with the values going from  $0.29 \text{ mg.g}^{-1}$  DW to  $0.35 \text{ mg.g}^{-1}$  DW in the leaves of the plants treated with 100 mM and  $0.39 \text{ mg.g}^{-1}$  DW when the salinity rose to 200 mM.



**Fig. 1. Variations in phenolic compound content ( $\text{mg.g}^{-1}$  PS) in the roots and leaves of plants from three species of bean: *Vigna angularis* L.(a), *Vigna mungo* L.(b), and *Vigna radiata* L.(c), under salt stress conditions.**

For *Vigna mungo* L. (Fig. 1b), the polyphenol content rose significantly from  $0.40 \text{ mg.g}^{-1}$  DW in the leaves of the control plants to  $0.48 \text{ mg.g}^{-1}$  DW with the treatment at 200 mM of NaCl. It should be noted that the leaves ( $0.46 \text{ mg.g}^{-1}$  DW) accumulated twice as many polyphenols as the roots ( $0.28 \text{ mg.g}^{-1}$  DW) in the plants stressed at 200 mM. For *Vigna radiata* L. (Fig. 1c), the root polyphenol content dropped with the increase in salinity, whereas in the leaves, these compounds had very low values in the salt milieu at 100 mM ( $0.3 \text{ mg.g}^{-1}$  DW); but when the salinity increased to 200 mM, the polyphenols accumulated significantly ( $0.41 \text{ mg.g}^{-1}$  DW).

### 3.2 EFFECT OF SALINITY ON THE FLAVONOID CONTENT OF THE ROOTS AND LEAVES

At the level of the roots in *Vigna angularis* L. (Fig. 2a), the accumulation occurred gradually as the milieu became enriched in salt. On the contrary, in *Vigna mungo* L. (Fig. 2b), there was a significant drop in flavonoids under the effect of both concentrations of NaCl in relation to the control plants (or  $1 \mu\text{g}\cdot\text{g}^{-1}$  DW versus  $0.29$  and  $0.32 \mu\text{g}\cdot\text{g}^{-1}$  DW). For *Vigna radiata* L. (Fig. 2c), flavonoid content remained very low in the roots of all the plants. At the level of the leaves, in *Vigna radiata* L. (Fig. 2c) the NaCl provoked an accumulation of flavonoids, up to a level of  $0.98 \mu\text{g}\cdot\text{g}^{-1}$  DW at 200 mM of NaCl; a slight reduction in these compounds was observed ( $0.8 \mu\text{g}\cdot\text{g}^{-1}$  DW). In *Vigna angularis* L. (Fig. 2a) and *Vigna mungo* L. (Fig. 2b) the flavonoid content increased clearly in the leaves with the salinity of the culture medium at 100 mM of NaCl ( $0.82$  and  $0.87 \mu\text{g}\cdot\text{g}^{-1}$  DW). At the higher concentration, the flavonoid content of the leaves dropped significantly in both species ( $0.50$  and  $0.62 \mu\text{g}\cdot\text{g}^{-1}$  DW).

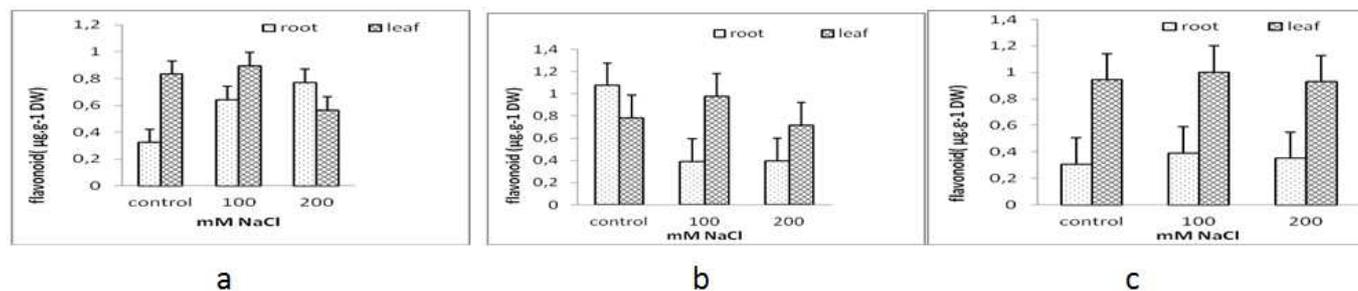


Fig. 2. Variations in flavonoid content ( $\mu\text{g}\cdot\text{g}^{-1}$  PS) in the roots and leaves of 3 species of bean: *Vigna angularis* L.(a), *Vigna mungo* L. (b), and *Vigna radiata* L. (c), under salt stress conditions.

## 4 DISCUSSION

Our results show that phenolic compound content decreases when a saline solution (NaCl) is applied to the roots; on the other hand, in the leaves it increases, particularly in *Vigna angularis* L. and *Vigna mungo* L. when subjected to salt stress at 200 mM. Our results are in agreement with those (11), on radish seedlings (12), on *Vetiveria zizanioides* L. (13). Other authors, reported an increase in phenols in a study of romaine lettuce (13). In other recent studies, we can also observe an increase in phenolic compound content under salt stress conditions, on okra (14), on *Cymbopogon nardus* L. (15) and on *Moringa oleifera* L. (16). We can also see that polyphenol content in the roots of the three species increased when treated with 100 mM of NaCl. Total phenolic compound content in three broccoli cultivars increased significantly when treated with 160 mM of NaCl (17). These increases in phenolic compounds are also reported in radishes subjected to 100 mM of NaCl whereas no response of the same nature was recorded with salinity at 10 and 50 mM of NaCl. (18)

The results recorded for flavonoid content in the leaves of the three species were significantly high with the treatment at 100 mM of NaCl; the same observations were made, on *Lactuca sativa* L. (19), as well as on a few tomato lines (20), beans (21) and artichokes (22).

## 5 CONCLUSION

Phenolic compounds and flavonoids are considered to be secondary metabolites in response to plants exposed to environmental stress. These compounds act as antioxidants to protect the plant's organs under stressful conditions. Salt stress may accelerate the synthesis of phenols, which depends considerably on the sensitivity to salt of the plants, the salt concentration and the plant cultivated. At the end of the present study to assess polyphenol and flavonoid content in three species of *Vigna* under salt stress conditions at 100 mM and 200 mM. I<sup>-1</sup> of nutrient solution We also observed accumulation at the level of the leaves compared to the roots. The results of these experiments have made it possible to note differences in species with regard to salinity. The *Vigna radiata* L. variety seems to be more sensitive to salt, whereas *Vigna angularis* L. shows greater tolerance.

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