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Anti-inflammatory potential of *Lupinus mutabilis* seeds: isolation of deflamin, a polypeptide with a potent inhibitory effect on MMP-9 activities

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INTRODUCTION

Matrix metalloproteinases (MMP) are a family of proteolytic enzymes responsible for degrading the components of the extracellular matrix(1). Gelatinases MMP-9 and MMP-2 are implicated in several important processes and diseases, such as Inflammatory bowel disease, Crohn's disease and colorectal cancer (2,3) and MMP inhibitors (MMPI) have been proven to positively influence some of these pathologies. Previous studies by our work group(4) show the presence of deflamin in *L.albus* seeds, a novel MMPI that has anti-inflammatory action, both *in vivo* and *in vitro*. Deflamin has a great nutraceutical potential, as it can withstand boiling and low pH levels. This study aims to assess the presence and activity of deflamin in *L. mutabilis* seeds.

MATERIALS AND METHODS

Biological material: Dry ripened seeds of 11 different *Lupinus mutabilis* lines were used. The lines were chosen in order to obtain a heterogenic sample pool and are the following: JKI-309, JKI-210, JKI-295, JKI-377, Mutal, LM 268, Potosi-ISA, Inti (late and early), PRT-79 and SBP. *Lupinus albus* var. *amiga* was used as control.

Bioactivity: MMP-9 inhibitory activity of total protein extracts of the different seeds was evaluated through reverse zymography and the DQ-gelatin method.

Deflamin isolation: Deflamin was isolated according to a method developed in our lab. The protein profile of the purified protein was evaluated through SDS-PAGE.

RESULTS

MMP-9 inhibitory activity in *L. mutabilis* lines Reverse zymography

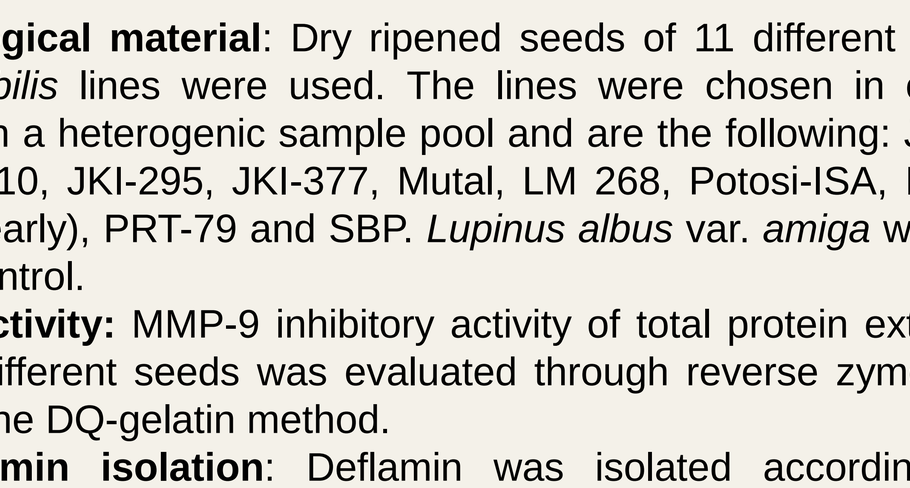

Figure 1: Gels obtained by performing a reverse zymography to the total soluble protein extract. The dark bands against the clear background indicate spots where the MMP-9 did not degrade the substrate (gelatin).

The reverse zymography (figure 1), a method that allows the detection of protease inhibitors(5), shows bands that indicate the presence of an MMPI, suggesting the presence of deflamin in higher amounts in JKI-210, JKI-295 and SBP.

MMP-9 inhibitory activity in *L. mutabilis* lines DQ gelatin method

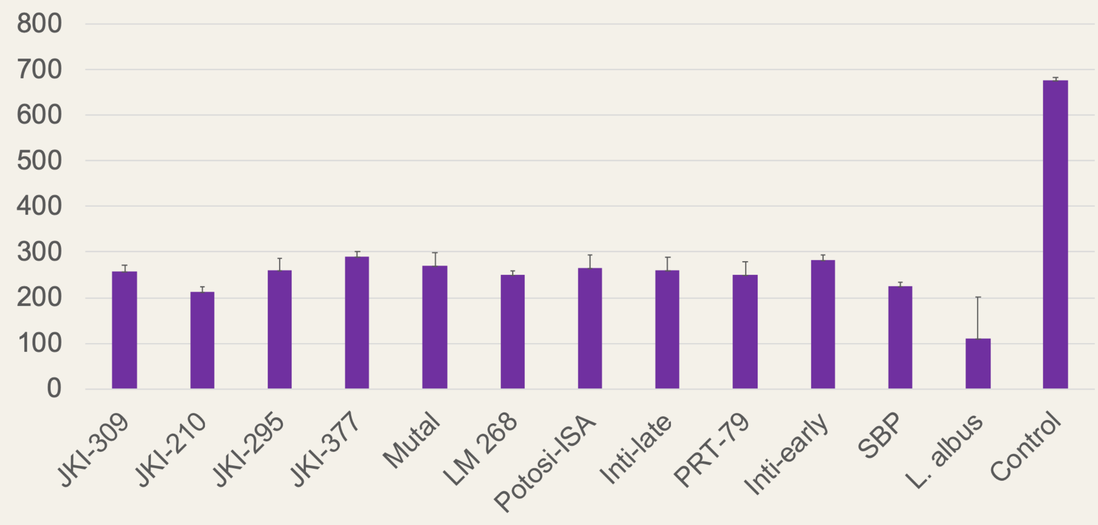

Figure 2: Effect of total protein extracts on the activity of comercial available MMP-9, obtained by measuring the fluorescence in a dye-quenching gelatin fluorometric assay

Gelatinolytic activity shows that lines JKI-210 and SBP are stronger inhibitors of MMP-9 activity

The presence of deflamin in *L. mutabilis* lines

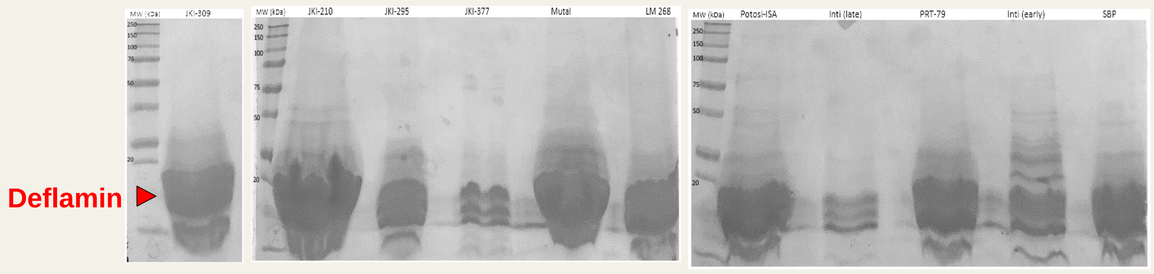

Figure 3: Polypeptide distribution of the isolated deflamin through SDS-PAGE.

Deflamin, a small (around 20kDA) polypeptide is present in all of the studied lines, but its amounts differ considerably.

CONCLUSIONS

- With these assays we were able to prove that deflamin, a low molecular mass gelatinase inhibitor, was present in all studied lines, but with different levels of activity and in different amounts.
- We can conclude that the seeds of *L. mutabilis* are strong candidates to be included in anti-cancer and anti-inflammatory diets and as nutraceuticals, in order to prevent and treat several ailments.

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