Doctoral Thesis

# Evaluation of the effect of some technological elements on the productivity and quality of some soybean varieties cultivated in northwestern Romania

## (THESIS SUMMARY)

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## Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the most important agricultural crops in the farming landscape of our country, as well as in Europe and many other regions around the globe.

Soybean harvests provide approximately **60% of the world's plant-based protein** (Muntean et al., 2014). The global market for soy-based protein ingredients (protein concentrates, soy protein isolates, textured soy proteins, soy flour, etc.) was valued at USD 7.7 billion in 2022 and is expected to grow to USD 10.8 billion by 2027, with a compound annual growth rate (CAGR) of 7.0% during the forecast period (2022–2027). This protein has a nutritional value comparable to that of animal-based foods. Soybeans also have important medicinal properties. Due to their particularly high nutritional value, soybeans are used in human nutrition, animal feed, as a phytotherapeutic source, and as a raw material in industry. In the United States, soybean production is primarily used as feed, especially defatted meal resulting from the oil extraction process and subsequent defatting (Ruiz et al., 2020).

The expansion of soybean cultivation areas is supported by several essential factors. Firstly, the global demand for plant proteins is increasing for both human and animal consumption, and soy provides one of the most complete protein sources in the plant kingdom (Hartman et al., 2011). Secondly, in the context of climate change and soil degradation, soy contributes to improving soil fertility through biological nitrogen fixation, reducing the need for chemical fertilizers (Salvagiotti et al., 2008). Additionally, soy-derived products (meal, oil, plant-based milk, tofu, biofuel) offer various industrial and food uses, supporting the development of diversified and sustainable value chains.
This doctoral thesis aims to highlight the role of artificial inoculation of soybean seeds with bacterial biopreparations on the yield and quality of three soybean varieties cultivated under the pedoclimatic conditions of northwestern Romania.

## Research Purpose and Objectives

The main objective of the research is to evaluate the effect of crop rotation and seed bacterization on soybean yield, productivity elements, and seed quality in three early soybean varieties (Onix, Felix, and Cristina TD), developed and certified at the Agricultural Research and Development Station (SCDA) Turda.

The specific objectives of the doctoral thesis were as follows:

- To assess the influence of crop rotation, seed inoculation, and soybean variety on grain yield and main productivity components.
- To determine the impact of crop rotation and seed inoculation on soybean seed quality indices, including protein and oil content.
- To analyze the combined effect of crop rotation and inoculation on morphological characteristics, yield, and productivity components:
    - Investigating how crop rotation and seed inoculation influence plant height and the insertion of the first pod in soybeans.
    - Determining the impact of the interaction between crop rotation and inoculation on grain yield, number of pods per plant, number of seeds, and their mass.

- Evaluating how the interaction between crop rotation and inoculation affects seed quality, including protein, oil, and fatty acid content (stearic, oleic, linoleic, linolenic).

- To analyze the differences among soybean varieties in response to crop rotation in terms of productivity and biometric characteristics:
  - Comparing the response of soybean varieties to different crop rotations in terms of plant height and first pod insertion.
  - Evaluating the impact of crop rotation on the productivity of each soybean variety analyzed.
  - Determining the effect of crop rotation on seed quality for each soybean variety.
- To investigate how different soybean varieties respond to seed inoculation regarding plant height, first pod insertion, yield, and productivity elements:
  - Comparing the effect of inoculation on plant height and first pod insertion across different soybean varieties.
  - Evaluating the effect of inoculation on grain yield and productivity elements for each soybean variety.
  - Determining how different inoculation variants influence protein, oil, and fatty acid content for each soybean variety.
- To analyze the relationships between productivity and seed quality in soybeans based on crop rotation and inoculation:
  - Identifying correlations between yield, productivity, and quality across various crop rotations.
  - Determining the relationship between productivity and quality based on the inoculation variant applied.
- To evaluate the yield stability of soybeans depending on variety, crop rotation, and inoculation to identify the variants with the best adaptability:
  - Analyzing the variability of soybean yield depending on crop rotation for each studied variety.
  - Determining the impact of inoculation on yield stability in each analyzed soybean variety.
  - Investigating the combined influence of crop rotation and inoculation on soybean yield stability.

## Methodology

To evaluate the influence of crop rotation and inoculant application on soybean seed productivity, productivity elements, and quality parameters in three early-maturing varieties, a field experiment was conducted in the locality of Amaţi, Satu Mare County, over two consecutive agricultural years (2020 and 2021).

The biological material studied consisted of three soybean varieties classified as early-maturing genotypes based on their vegetation period (number of days). These were developed and approved at SCDA Turda, with the Onix variety registered in 2002 and the Felix variety in 2005. Cristina TD is a more recent variety created in Turda, notable for its high thousand-seed weight (TSW) and the light color of its hilum (yellow, matching the seed coat), which makes it suitable for food industry use. Similar characteristics meeting food industry requirements are found in the early variety Felix, which not only has good protein content in the seed but also a light-colored hilum (light

gray). Onix is a widely cultivated soybean variety in Romania, known for its high ecological plasticity.

The experiment was multifactorial and was organized according to a split-plot model, including three factors:

Crop rotation, with two levels: A1 - Soybean introduced for the first time in rotation (after four years of forage crops); A2 - Soybean included in a five-year rotation (Control).

Seed inoculation with bacteria, with three levels: I1 - Control (no inoculant); I2 - Poliriz (treatment with Poliriz S); I3 - Nitragin (treatment with Nitragin).

Soybean variety, with three levels (the variety average was considered as control): S1 - Onix; S2 - Felix; S3 - Cristina TD.

**Laboratory Methods**. At the end of the vegetation season, 10 plants from each experimental variant were destructively analyzed for the following parameters: plant height, first pod insertion, number of pods per plant, number of seeds per plant, seed mass per plant, and seed size (TSW - thousand seed weight). Yield was estimated based on the thousand-seed weight, number of seeds per plant, and plant density per $m^2$ at full maturity.

Using NIR spectroscopy (Tango, Bruker Optik GmbH, Ettingen, Germany), three samples of unground seeds from each experimental variant were analyzed in the laboratory for: protein content; oil content; stearic acid content; oleic acid content; linoleic acid content; linolenic acid content.

Experimental data were processed using variance analysis with the Polifact software, and the results were represented through matrix-type graphs using Past4 software, as well as Excel-generated charts. The matrix graphs display values through color maps, where cooler shades represent lower values, and warmer shades represent higher values. The Pearson correlation coefficient matrix was developed in Past4, and yield stability was calculated using favorability indices based on average values in Excel.

## Results and Discussion

### Variance Analysis of Yield, Main Agronomic Traits, and Quality Indices in Three Early Soybean Varieties

Based on the F-test values from the variance analysis, both plant height and the insertion of the first pod were highly significantly influenced by the variety factor.

Among the technological factors analyzed, only inoculation had a distinctly significant influence on the variation in plant height among the three soybean varieties studied under two different crop rotation systems. Additionally, the insertion height of the first pod was significantly affected by the interaction between crop rotation and variety, highlighting the impact of these factors on plant architecture under the experimental conditions.

### Influence of the Interaction Between Inoculation and Crop Rotation on Biometric Traits, Yield, and Productivity Elements

In the Poliriz-inoculated variant, plant height increased by 3.5 cm in rotation A1 and 3.7 cm in A2, with both increases being statistically significant ($p < 5\%$). These values are shown in the graph with a yellow shade, indicating an intermediate growth level.

The strongest influence was observed with Nitragin inoculation, where plants exhibited an average height increase of 5.33 cm in A1 and 5 cm in A2, differences that were highly statistically significant ($p < 1\%$). These values are shown in warm colors (orange-red), indicating greater plant height. Nitragin inoculation had the most

pronounced effect on height growth in soybeans, while the rotation type had a secondary yet slightly favorable influence when combined with biological treatments. These results underscore the importance of inoculation in soybean cultivation technology for producing vigorous plants.

Regardless of the inoculant type used (Poliriz or Nitragin) and the crop rotation (A1 or A2), the changes observed in first pod insertion height did not exceed the thresholds of statistical significance (LSD 5%, LSD 1%).

The number of pods per plant ranged from 35.3 to 37.3 depending on crop rotation and inoculation. In general, the number of seeds per plant varied considerably depending on inoculation and rotation. Medium to high values, indicated by orange coloring, were observed in the rotation where soybeans were newly introduced, with both the minimum (83) and maximum (99.5) values of the experiment recorded under the five-year rotation. Regardless of the crop rotation studied, seed inoculation led to a higher number of seeds per plant, with the most substantial increase observed with the use of Nitragin.

The seed mass per plant varied significantly, ranging from 13.4 g to 16.2 g depending on rotation and treatments applied. The highest values were recorded in the rotation where soybean was introduced for the first time. Moreover, seed inoculation prior to sowing had a positive effect on seed mass, contributing to better results compared to non-inoculated variants.

The thousand-seed weight (TSW) varied within a relatively narrow range, from 158 g to 170 g, depending on rotation type and treatments applied. The highest values were obtained in the variant where soybeans were inoculated with Poliriz and newly introduced into rotation, suggesting a synergistic effect between inoculation and rotation on this production element.

Conversely, the lowest TSW values were recorded in the non-inoculated variant under the five-year rotation, highlighting the essential role of symbiotic bacteria in ensuring good plant and seed development. Even in this case, inoculation led to an increase in seed mass, though differences were not always statistically significant, indicating the influence of additional factors such as pedoclimatic conditions or genetic characteristics of the variety.

The protein content of soybean seeds varied only slightly depending on the technological sequences studied, ranging from 38.8% to 39.2%. The maximum value was obtained when soybean was newly introduced in the rotation and seeds were not inoculated.

On average, over the two experimental years, the oil content of the three soybean varieties ranged from 19.3% to 19.7%, with the maximum value observed when soybeans were included in a five-year rotation and seeds were not inoculated with nitrogen-fixing bacteria before sowing.

From the influence of the dual interaction between inoculation and rotation on stearic acid content, it is evident that inoculation resulted in lower values regardless of rotation type, with the minimum value of 4.53% recorded in soybeans included in a five-year rotation and treated with Poliriz. Values near the maximum (4.76%) or the highest were observed in non-inoculated soybeans or those treated with Nitragin when newly introduced in rotation.

**Influence of the Interaction Between Variety and Crop Rotation on Biometric Traits, Yield, and Productivity Elements**

The dual interaction between variety and rotation on plant height shows that the Onix variety recorded the highest plant height values, exceeding 125 cm in both types of rotation. The differences from the control were highly statistically significant (***), indicating lush growth compared to the variety average.

On the other hand, the Felix variety had the smallest plant height values, ranging from 102–106 cm. It was below the variety average in both rotations, with very significantly negative differences (000), making it the variety with the shortest stature in the experiment.

Cristina TD exhibited an intermediate height (~118 cm), exceeding the variety average in both rotations, with statistically significant differences (** for A1 and * for A2).

Soybean yield in the experiment varied according to the genetic factor and the rotation selected, ranging from 4,137 kg/ha (Felix in rotation where soybean was included) to 5,188 kg/ha (Cristina TD, in rotation where soybean was introduced for the first time). On average, in rotation A1, a yield increase of 358 kg/ha was recorded compared to A2. In both rotations, the Cristina TD variety demonstrated very good productivity, achieving significantly positive yield increases compared to the variety average (figure 1).

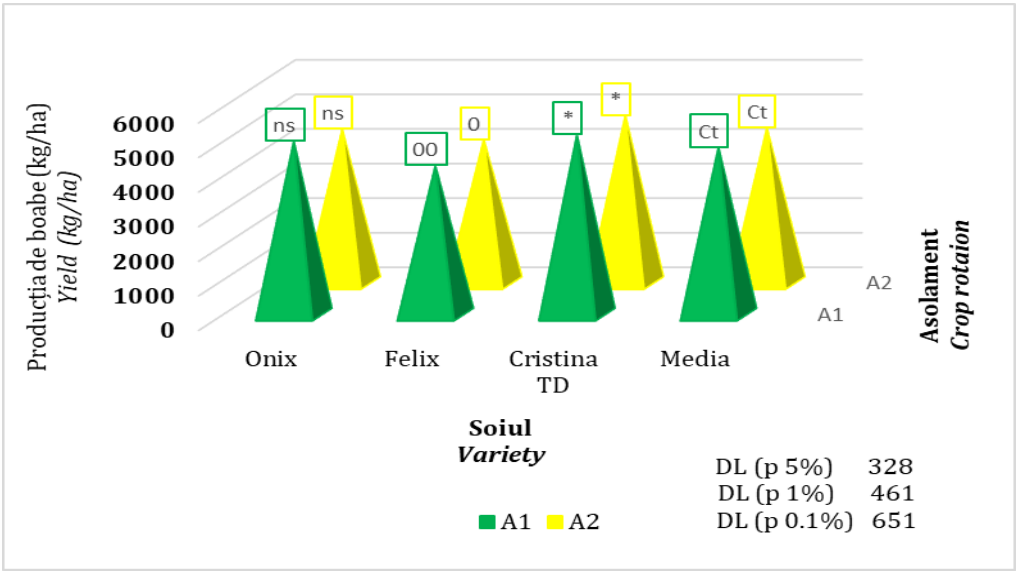


Fig. 1. **Influence of the Dual Interaction Between Variety and Crop Rotation on Yield**

Although the number of pods per plant is an important yield component, it does not always correlate positively with total yield. This is evident in the current experiment, where the most productive variety, Cristina TD, recorded significantly negative differences (A2) and distinctly significantly negative differences (A1) compared to the variety average. For the Felix variety newly introduced in rotation, the number of pods per plant was about 30, six fewer than the average of the three varieties.

The number of seeds per plant varied considerably under the interaction between variety and rotation.

Once again, the Onix variety stood out, recording very significantly positive differences compared to the variety average, regardless of rotation. It had 104 seeds/plant (A2) and 111 seeds/plant (A1).

At the opposite end was the early soybean variety Felix, which recorded statistically assured very significantly negative differences (A1) and distinctly significantly negative differences (A2) in terms of seeds per plant compared to the average of the three experimental varieties.In the current experiment, the protein content of the three soybean varieties depending on rotation ranged from 37.91% (Onix, newly introduced in cultivation) to 39.95% (Cristina TD, in the same rotation) (Fig. 2).

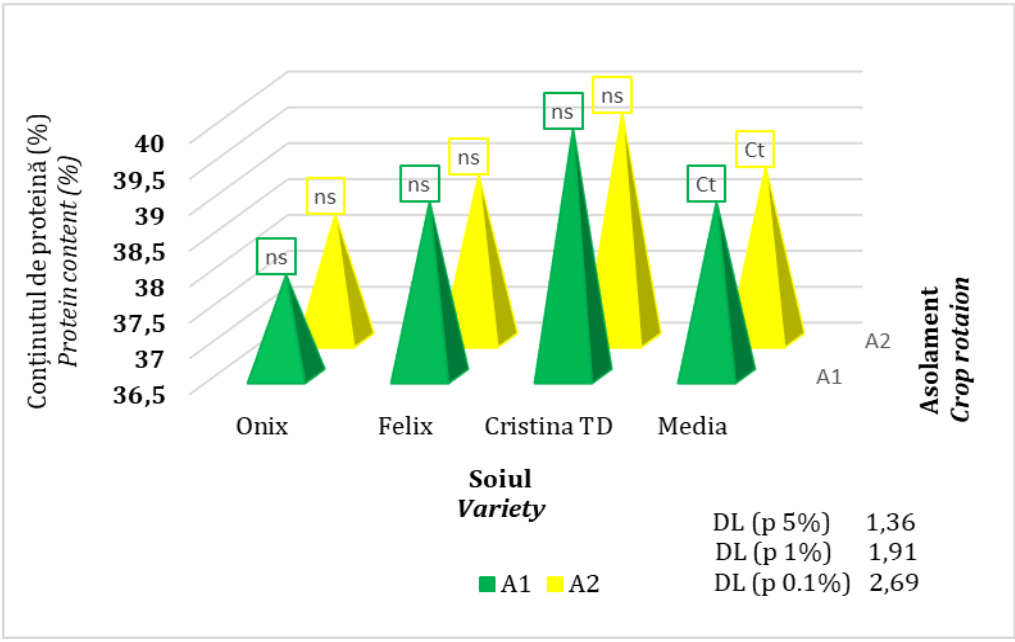


Fig. 2. **Influence of the Dual Interaction Between Variety and Crop Rotation on Protein Content**

Once again, Cristina TD stood out not only for high yield and large seed size but also for its high protein content, making it one of the recommended varieties for food industry use.

On average, slightly higher stearic acid content values were observed in the rotation where soybeans were newly introduced compared to the variant where they were part of a five-year rotation. Regarding the behavior of the three varieties, the differences obtained were statistically insignificant compared to the average of the three varieties, regardless of rotation.

The Onix variety stood out for the highest oleic acid content in both rotations, with a significantly positive difference from the average in the rotation where soybean was newly introduced. The average oleic acid content varied more by variety than by rotation.

Cristina TD showed the lowest values of this oil quality indicator, with statistically assured significantly negative differences compared to the average of the three varieties, in both rotations.

The stearic acid content averaged 24.66% when soybeans were part of a five-year rotation and 24.52% when newly introduced in rotation.

For both Felix and Onix varieties, the differences obtained in linoleic acid content were not statistically significant, regardless of whether soybean was newly introduced or included in a five-year rotation. The results emphasize that the fatty acid profile can be improved more effectively through breeding by creating valuable genotypes than by applying a specific cultivation technology.

For the Cristina TD variety, seed treatment with Nitragin resulted in greater plant height, with a significantly positive difference compared to the control.

The dual interaction between inoculation and variety on the number of pods per plant shows that this key productivity component varied more by variety than by inoculant treatment. Nevertheless, a slight increase was observed following Poliriz and Nitragin treatments, regardless of the variety studied. The Felix variety, when seeds were not inoculated, had the lowest number of pods per plant, whereas the experiment's maximum of 45 pods per plant was observed in the Onix variety treated with Nitragin. The Cristina TD variety also responded well to seed treatment with Nitragin. Although small differences were observed with inoculation, they were not statistically significant.

The best response to Nitragin inoculation was observed in the Onix variety, which achieved the experiment's maximum of 114 seeds per plant. In contrast, the Felix variety, without inoculation, had only 77 seeds per plant. A positive reaction to both Poliriz and Nitragin treatments was also noted in Cristina TD, which showed higher values than the control in these cases. While inoculants appeared to increase the number of seeds per plant, the differences were not statistically assured.

Although Onix ranked highest in productivity elements such as pod and seed number per plant, it had the lowest thousand-seed weight (TSW) values (Fig. 3). The range of variation in TSW by variety and inoculation was 38 g, ranging from 142 to 180 g. The smallest seeds were observed in Onix without inoculation and with Nitragin treatment. Conversely, Poliriz application led to high TSW values for both Felix and Cristina TD. Cristina TD also responded well to Nitragin. In general, higher TSW values were recorded in the Poliriz-treated variants.

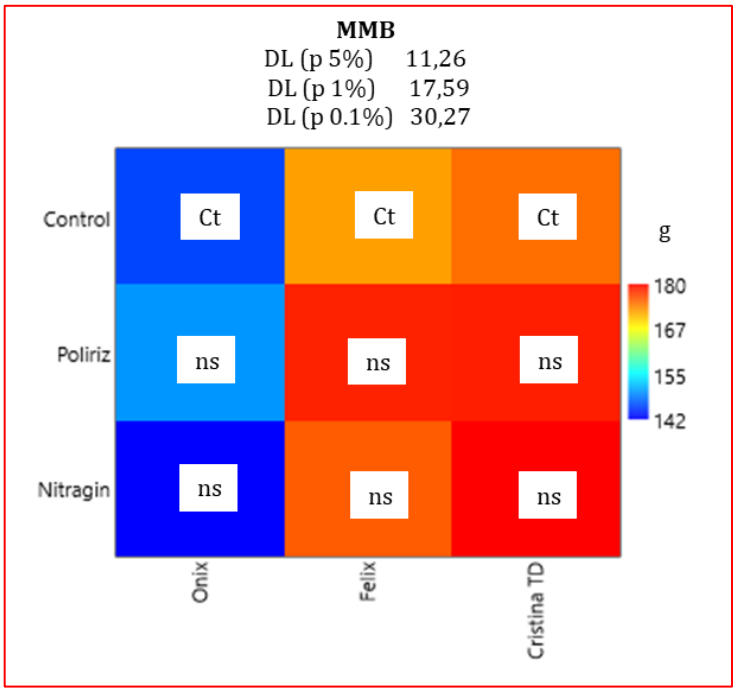


Fig. 3. **Influence of the Interaction Between Inoculation and Variety on Thousand-Seed Weight (TSW)**

**Protein Content and Inoculation**

Non-inoculated soybean seeds had significantly lower protein content compared to inoculated seeds, according to Zimmer et al. (2016). Vollmann et al. (2000) showed that nitrogen fertilization during flowering led to higher seed protein content than both the control and Rhizobium-inoculated variants. However, given the complex nature of biological nitrogen fixation, protein content variation may be influenced by multiple genetic, environmental, and technological factors as well as their interactions—highlighting the need for further research.

**Conclusions**

The evaluation of crop rotation and seed bacterization effects on soybean yield, productivity elements, and seed quality in three early soybean varieties (Onix, Felix, and Cristina TD) allowed for drawing meaningful conclusions for both soybean growers and researchers.

Both plant height and first pod insertion were highly significantly influenced by the variety factor. Among the technological factors analyzed, only inoculation had a distinctly significant effect on plant height variation across the three soybean varieties studied under two different crop rotations. Furthermore, first pod insertion was significantly influenced by the interaction between crop rotation and variety, highlighting the impact of these factors on plant architecture under the given experimental conditions.

Protein content was significantly affected by the same factor, whereas quality indices fluctuated less, without statistical assurance, based on the crop rotation or type of inoculation practiced.

Inoculant treatments significantly increased plant height compared to the control (no inoculation), regardless of the crop rotation applied. In the Poliriz-inoculated variant, plant height increased by 3.5 cm in A1 and 3.7 cm in A2, with both increases being statistically significant ($p < 5\%$). These values are shown in the graph with a yellow hue, indicating an intermediate level of growth.

The most pronounced effect was seen in the Nitragin-inoculated variant, where plants grew an average of 5.33 cm in A1 and 5 cm in A2—differences that were highly statistically significant ($p < 1\%$).

Seed inoculation with Poliriz or Nitragin changed the negative correlation between protein and TSW into a positive one, with correlation coefficients of 0.37 and 0.38, respectively. Although the values are not high, inoculation could be considered a viable solution for obtaining large, protein-rich seeds suitable for use in the food industry.

Soybean exhibited pronounced stability, regardless of treatment, when cultivated for the first time on a plot after four years of forage crops. Under this rotation, yields were not only significantly higher but also more stable—this stability being described by a regression line nearly parallel to the X-axis. Once again, the superiority of variants in which seeds were inoculated on the sowing day with Poliriz and Nitragin is evident, with the latter showing particularly strong results.

**Originality and Innovative Contributions of the Thesis**

This thesis is original in its aim to evaluate the effect of crop rotation and seed bacterization on the yield of three varieties developed at SCDA Turda, well-established in Romanian agriculture (Onix, Felix, and Cristina TD). It highlights differences in productivity elements and seed quality among experimental variants (different rotations, different bacterial inoculation treatments, and genetic variety).

The originality of the doctoral thesis also derives from the combination of the analyzed technological elements and the specificity of the crop area in which the experiment was located. The use of two types of biological inoculants brings an added novelty, and their integration in the context of crop rotation and the evaluation not only of the production capacity and its stability depending on technological factors, but also of the quality of the grains, contributes to the relevance and practical applicability of the research.

Another element of originality is the potential contribution to reducing the carbon footprint in agriculture, as the use of biological inoculants favors biological nitrogen fixation, reducing the need for chemical fertilizers and implicitly the emissions associated with their production and application. Thus, the thesis has not only scientific and agronomic value, but also ecological, by highlighting sustainable practices adapted to local pedoclimatic conditions.

The results obtained have immediate applicability in Romanian crop farms and beyond.