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Influence of *Colletotrichum truncatum* on the Physiological and Chemical Quality in Different Varieties of Soy Seed

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Abstract

The literature highlights that a severe infection by the fungus *Colletotrichum truncatum* may be capable of inflicting considerable damage to seeds after harvest, potentially affecting their chemical composition and physiological quality. Taking into account that currently there is no categorization in terms of susceptibility and tolerance on this pathogen, the present work is presented with the main objective of "Evaluate the influence of Pathogenicity of *C. truncatum* on the physiological quality (germination, vigor, viability) and biochemical components in different varieties of soybean seeds (*Glycine max*)" most planted in the region. The work was carried out in the Agrotec laboratory, located in the Municipality of San Alberto (Alto Paraná), using a completely randomized experimental design, with AxB factorial arrangement, where A indicates ten most planted soybean varieties in the region and B with or without artificial inoculation of *Colletotrichum truncatum*, with twenty treatments and four repetitions. The variables evaluated were: germination, vigor, viability and chemical composition. The data were subjected to analysis of variance and the Tukey test at 5% error. The results showed a significant statistical difference, accepting the alternative hypothesis proposed "The pathogenicity of *Colletotrichum truncatum* influences the physiological quality (germination, vigor, viability) and biochemical components (saturated and unsaturated fatty acids) in different varieties of soybean seeds (*Glycine max*)".

Keywords

Colletotrichum truncatum, Physiological Quality, Chemical Composition, Soybean Varieties (*Glycine max*)

1. Introduction

Soybean is an important oilseed crop in the world due to its high protein and oil content in the seed for animal and human consumption, contributing 3.3% of global human caloric intake [1]. It is one of the most important crops for the country, being the most exported internationally, contributing considerably to the Paraguayan economy. During the last decades, the production of soybean cultivation has become a challenge for producers since there are a series of adversities that they must combat, among them are pathogenic agents, since they are a major problem for agricultural technology, which has responded with the launch of various genetically improved varieties according to the needs of farmers, in addition to agricultural defensives that could alleviate the day-to-day problems in the field in order to obtain good yields accompanied by grain quality [2].

It is known that diseases are among the main factors that can affect soybean production; among them, there is the case of *Colletotrichum truncatum*, commonly called anthracnose, a highly harmful fungal disease, which is becoming a great threat in production areas around the world [3], and which can occur throughout the vegetative and reproductive cycle causing great losses, both in grain quality and yield [4] and for which there is no categorization in terms of susceptibility and tolerance on *C. truncatum* in the soybean seed varieties commonly marketed in the region.

Fungi have a great capacity to infect all components of the seed, with the greatest incidence in the cover, followed by the cotyledons and embryonic axes without any apparent external perceptible symptom, visualized in the decrease in vigor and viability due to the deterioration of the seed. However, it is precisely the vigor and viability of the seeds that are important characteristics for the establishment and maintenance of a uniform stand of seedlings, since the higher the physiological quality of the seed (viability and vigor), the better the establishment of seedlings is obtained healthy under a wide range of environmental conditions [5].

Protein constituents with essential amino acids and oils as a rich source of plant fatty acids serve as a nutrient medium for the establishment, survival and multiplication of a wide range of pathogenic fungi [6]. Soybean oil contains a lower amount of saturated fats (palmitic and stearic acid) and a higher amount of unsaturated fatty acids (oleic, linolenic and linoleic acid), highly desirable in the human diet. However, it has been shown that soybeans infected with fungi can also cause chemical decomposition of their protein constituents and oils [7].

In a research carried out by [8], Anthracnose caused by *Colletotrichum truncatum* is associated as one of the most important fungal pathogens transmitted by soybean seeds, resulting in a significant reduction in the physiological quality of the seeds such as germination, viability and vigor, in addition to the deterioration of biochemical qualities due to the change in nutritional profile such as protein content and fatty acid composition, decreasing its value as a grain and food.

Based on what was established, the main objective of this work was to evaluate the influence of pathogenicity of *Colletotrichum truncatum* on the physiological quality (germination, vigor, viability) and chemical composition (saturated and unsaturated fatty acids) of different soybean seed varieties.

2. Materials and Methods

The experiment was carried out in the Agrotec Laboratory, located in the Municipality of San Alberto, department of Alto Paraná, Paraguay with the following coordinates 24˚58'0''S, 54˚54'W.

2.1. Source of Soybean Seed Varieties Used

In the present investigation, soybean seeds from ten most used varieties with the greatest affinity by producers in the Canindeyú and Alto Paraná region were chosen.

Among them M5947 IPRO, M5705IPRO and M64I61 IPRO characterized by their high productive potential, early stability, aggressive root system and good health.

NS6483 and NS6906IPRO; both with high productive potential at early sowing dates, in addition to having optimal branching power, stable and rustic in lower fertility environments, resistant to diseases such as stem canker; while the NA5909 RR, characterized by its precocity, with high productivity and the possibility of staggering sowing, high stability in different environments, in addition to being resistant to diseases such as frog's eye spot, macrophhomina and phytophthora.

DM62R63; good stability and excellent yield potential, strong behavior against overturning since it has good vegetative bearing and adapts to different environments.

SOJAPAR R49, highly productive in areas where there is not good fertility, resistant to stem canker and Asian rust.

The 59I60 RSF IPRO - BMX DELTA IPRO characterized by its high performance, branching, precocity and health.

HO Pirapó (64HO114 IPRO), which stands out for having the highest performance potential within the product portfolio on the market. It has an adaptation for all regions of the country and is more compact, with short internodes; That is, it has no problems with tipping over.

2.2. Fungal Isolation and Seed Inoculation

The pathogen *Colletotrichum truncatum* was isolated from soybean seeds with signs of anthracnose and they were inoculated in potato dextrose agar (PDA) culture medium for multiplication, developing in approximately 5 to 7 days. Isolation was carried out by streaking the surface of Petri dishes, incubated at a temperature of 28˚C. The spores of the fungus were taken from the culture medium and mixed and homogenized in 15 ml of sterile distilled water, and then filtered through cheesecloth to eliminate the agar or remains of mycelium and brought to a final volume of 100 ml. Several counts were carried out in a Neubauer chamber by taking a drop with a Pasteur pipette, releasing an average of 3×10^4 spores per ml. The spore suspension obtained from the pure culture was adjusted to a concentration of 1×10^4 spores ml^{-1}, the concentration used for the inoculation of the experiments; using the formula $C_1 \times V_1 = C_2 \times V_2$, considering C_1 as the initial concentration (known in the count) V_1 = initial volume (established arbitrarily when preparing the inoculum) C_2 = desired final concentration V_2 = final volume (unknown).

2.3. Preparation of Inoculated Seeds and Control

Soybean seeds intended for inoculation and control were surface sterilized with 10% chlorine for 3 min. They were subsequently rinsed three times with sterile distilled water and allowed to dry for 1 h under a laminar flow chamber.

The seeds intended for infection were immersed for 1 h in a spore suspension (1:2 w/v) of *Colletotrichum truncatum*, followed by drying for 3 hours.

2.4. Experimental Design

The experiment was constituted by a factorial arrangement A × B (**Table 1**) where A indicates with and without artificial inoculation of *Colletotrichum truncatum* of soybeans and B ten varieties of soybeans arranged in a completely randomized design, with twenty treatments and four repetitions, totaling 80 units experimental, distributed completely randomly. Each experimental unit consisted of 100 seeds, which were sown on germitest paper, measuring 28 × 38, left in Germination ovens with 80% humidity at 25˚C - 26˚C, suitable for good

Table 1. Treatments used.

Treatments	Factor A	Factor B
		Variety
T1		SOJAPAR R49
T2		64I61SRF IPRO
T3	**Without inoculation**	62R63 RSF
T4		NA 5909 RG
T5	**With inoculation**	NS 6483
T6		64 HO114 IPRO
T7		M 5947 IPRO
T8		M 5705 IPRO
T9		NS 6906 IPRO
T10		59I60 SRF IPRO

development of a seedling, in accordance with the established by ISTA (International Seed Testing Association) rules.

2.5. Variables to Analyze

The physiological quality for both the controls and the inoculated ones was recorded 6 to 8 days after being placed in the oven on germitest paper. Therefore, the inoculated seeds were incubated for 6 - 8 days after artificial inoculation by *C. truncatum* following the above procedure and considered as infected seeds for this study.

2.6. Germination Percentage (PG)

To carry out the germination test, the methodology and criteria indicated by ISTA (2016) were used.

The substrates (germitest paper) containing the seeds were kept inside closed boxes, wrapped in plastic bags or placed directly on trays in the germination chamber, taking care that the relative humidity of the site could be maintained very close to saturation. During the permanence of the substrates, the necessary care was taken to ensure that they will not dry out and that they contain sufficient moisture for the entire analysis period.

Once the stipulated time (8 days) for the analysis arrived, the paper rolls were removed from the germinator for reading and evaluation, where the seedlings were classified into; normal seedlings (PN), abnormal seedlings (PA), hard seeds (SD), dead seeds (SM), fresh seeds (SF). The results were recorded in the Internal Germination Bulletin, expressed as a percentage, considering that the sum of the percentages of PN and PA and SD, SF and SM must give 100%.

2.7. Vigor Percentage (VP)

For this evaluation, the Accelerated Aging (EA) methodology indicated by [9] was followed for a period of 48 hours. This laboratory technique is composed of 2 well-defined stages where the first step was to subject the seed to a period of stress at high temperature and relative humidity and a second period of germination under optimal conditions. The test lasts 7 days for EA (accelerated aging).

For the stress period, 11.0 × 11.0 × 3.5 cm gerbox boxes were used that were clean and disinfected with alcohol. Inside each box, 40 ml ± 1.0 ml of water was placed, then the grids were placed inside and the soybeans on top of them, where they were distributed in such a way that they did not overlap. To finish the process, the boxes were covered and transferred to the Biochemical Oxygen Demand (BOD) at a temperature of 41˚C ± 0.3˚C for 48 h ± 15 minutes.

During the period that the boxes were in the BOD, care was taken to ensure that the seeds did not come into contact with water, and that there was a space of approximately 2.5 cm between the plates to guarantee uniformity in temperature distribution.

After 48 hours, sowing was carried out on germitest paper, where the methodology is the same as that used in the PG evaluation, leaving the germitest rolls inside the germinator for 5 days until the evaluation. During the time that the substrates were in the germination chamber, the temperature control of the germination chambers was carried out, recorded for the control bulletin. The evaluation of the seedlings, the calculations and the issuance of results were carried out following the criteria established for the *Glycine max* germination test.

2.8. Seed Viability (V)

This variable was evaluated using the Tetrazolium salt test (TZ), which basically consists of hydrating the seed to generate its physiological activity (respiration). The function of the tetrazolium salt will allow living cells to stain a reddish hue, which will indicate potential germination capacity. So, dead cells will not be stained.

In order to expose the embryo to the direct action of tetrazolium, the seed was subjected to a longitudinal incision, with subsequent staining of these, which were immersed in a solution of 2,3,5-triphenyltetrazolium chloride (tetrazolium) concentration. 0.75%, for which 75 ml of stock solution is diluted in 925 ml of water.

The samples were packed in previously moistened germination paper and pre-conditioned for 16 hours at 25˚C in the germinator, so that they can absorb water. After the immersion time had elapsed, the seeds were separated from the solution using a small strainer and washed previously with distilled water to remove excess dye [10].

For the evaluation, a cut was made in the soybean seed longitudinally crossing the embryonic axis to expose the central cylinder, removing the integument and observing the external and internal surface of the cotyledons to determine possible damage to the seeds that may be from mechanical damage, moisture damage and bed bug damage. These damages are represented by the type and level of staining which are classified into eight levels. From level five onwards, the seed will be considered unviable; in the event that there is an absence of red color, this will indicate lower viability or death of the embryo.

2.9. Chemical Composition (CQ)

The samples of inoculated and non-inoculated seeds were ground to obtain fine flour, and then sent to the Inpasa S.A laboratory, to carry out the oil extraction using the distillation method with petroleum ether. This methodology consisted of extracting three grams of each sample, and transferring them to a 10 × 10 cm qualitative paper for distillation in the distiller with petroleum ether, a process that lasts 6 hours until obtaining 100% pure oil. Once the oil was extracted from the sample, the analysis was carried out by gas chromatography for the evaluation of esters, where the percentage of saturated and unsaturated fatty acids and the influence of the pathogen *C. truncatum* on the chemical composition of the seed were observed.

2.10. Statistical Analysis

The data obtained were statistically analyzed with the analysis of variance (ANAVA) for which the use of the SAS statistical software was used [11]. The comparison of means was carried out using the Tukey test at 5% error.

3. Results

3.1. Germination Percentage (GP)

The results of the percentage of germination of soybean seeds are seen in Table 2, which shows that there was a highly significant difference according to the analysis of variance between the treatments in relation to this variable.

In (factor A) it was observed that the seeds without inoculation presented a higher percentage of vigor of 55% and there was a significant difference in relation to the seeds that were inoculated, with a difference of 14.4% at less vigor.

In relation to inoculation (factor B), a significant difference has also been presented. The SOJAPAR R49, NS 6483 and NS6906 IPRO varieties obtained better performance compared to the other varieties.

3.2. Viability Percentage (V)

The results of the percentage of viability of the soybean seed varieties are seen in Table 3. It is observed that there was no significant statistical difference according to the analysis of variance between the treatments in relation to this variable; however, in factor B it was observed that some varieties obtained a low percentage of viability.

Table 2. PG.

Germination percentage (%)			
Factor A		**Factor B**	
Without inoculation	55 a	SOJAPAR R49	88.1 a
Wit6h inoculation	40.6 b	NS 6483	84.7 ab
		NS 6906 IPRO	83.2 b
		62R63 RSF	67.8 c
		64I61 SRF IPRO	47.5 d
		64HO114 IPRO	46.1 d
		59I60 SRF IPRO	33.5 e
		M5705 IPRO	27.5 f
		M5947 IPRO	0.00 g
		NA 5909 RG	0.00 g
		DMS (5%) = 4.1273	

Values of the means followed by the same letters do not differ statistically from each other according to the 5% Tukey test.

Table 3. Viability of soybean seed varieties (V).

Viability			
Factor A		**Factor B**	
Without inoculation	82.3 a	SOJAPAR R49	98 a
With inoculation	82.3 a	NS 6483	97.2 a
		NS6906IPRO	96 a
		64I61SRF IPRO	94.4 a
		64HO114 IPRO	81.5 a
		59I60SRF IPRO	80.5 a
		62R63 RSF	80.5 a
		M5947IPRO	78.2 b
		M5705IPRO	72 c
		NA5909RG	44.7 d
		DMS (5%) = 4.3823	

Values of the means followed by the same letters do not differ statistically from each other according to the 5% Tukey test.

3.3. Chemical Composition of Seeds

The results of the evaluation of esters in the soybean seed are shown in **Table 4**. It is observed that there was a significant difference for both Factor A and Factor B according to the analysis of variance between the treatments in relation to this variable.

4. Discussion

The results of the percentage of germination and vigor of soybean seed varieties recorded that the seeds without inoculation presented a higher percentage of germination and vigor compared to the varieties without inoculation. In an experiment carried out by [12] they found that there was a significant difference between the varieties inoculated with *Colletotrichum truncatum* where a lower percentage of germination was observed in the seeds infested with said pathogen. Another similar experiment conducted by [13]. observed that the genus *Colletotrichum truncatum* was the one that had the lowest incidence in soybean seeds in comparison to the germination percentage in relation to other pathogens such *as Aspergillus spp*, *Penicillium spp*, *Phomopsis spp*, *Cercosporakikuchii*, *Cladosporium spp*. [14], in their experiments carried out, they highlight the health of the seeds, since a seed infected with pathogens reduces its vigor by up to 30%.

Regarding the Viability of the soybean seed varieties, although there was no significant statistical difference, the inoculated seeds showed lower values. A similar experiment carried out by [8] demonstrated contradictory results in the viability variable since the results obtained showed that the pathogen *C. truncatum*

Table 4. CQ chemical composition (saturated and unsaturated fatty acids).

Chemical composition				
Factor (A)	**Unsatured**	**Factor (B) Variety**	**Insatured**	**Satured**
Without inoculation	3.4 a	SOJAPAR R49	6.9 a	15.3 a
With inoculation	0.9 b	64I61SRF IPRO	2.2 b	1.8 d
		62R63 RSF	1.7 c	8 b
Factor (A) insatured	Satured	NA 5909 RG	1.7 c	7.7 b
With inoculation	9.5 a	NS 6483	1.6 d	2 d
Without inoculation	2.2 b	64 HO114 IPRO	1.5 e	2.8 d
		M 5947 IPRO	1.53 f	9.4 b
		M 5705 IPRO	1.51 f	3.2 cd
		NS 6906 IPRO	1.4 g	6.3 bc
		59I60 SRF IPRO	1.3 h	2.1 d
			DMS (5%) = 0.0387	DMS (5%) = 3.2952

Values of the means followed by the same letters do not differ statistically from each other according to the 5% Tukey test.

did affect the viability of the seeds, reducing their viability significantly.

The seeds of the soybean varieties SOJAPAR R49 and NS 6483 showed better physiological quality in terms of PG, PV and V compared to the other varieties when they were subjected to inoculation of the pathogen *C. truncatum*, evidencing a possible tolerance to it.

In reference to the chemical composition of the soybean seed varieties, those inoculated had alterations in both saturated and unsaturated fatty acids, thus demonstrating that the pathogen *C. truncatum* alters the chemical composition since it increases the amount of saturated fatty acids in relation to seeds that were not inoculated.

In factor B, it was observed that there was a significant difference between the varieties where some varieties showed a greater amount of unsaturated fatty acids, indicating that they have higher quality in the chemical composition where the variety that stood out the most is Sojapar R49 with 6.9% (**Figure 1**), in the blue bars correspond to saturated fatty acids and the red bars correspond to unsaturated fatty acids.

In a similar experiment carried out by [8], they demonstrated that *C. truncatum* alters the chemical composition of the seeds since the batches infected with said pathogen had an increase in the content of unsaturated fatty acids and a

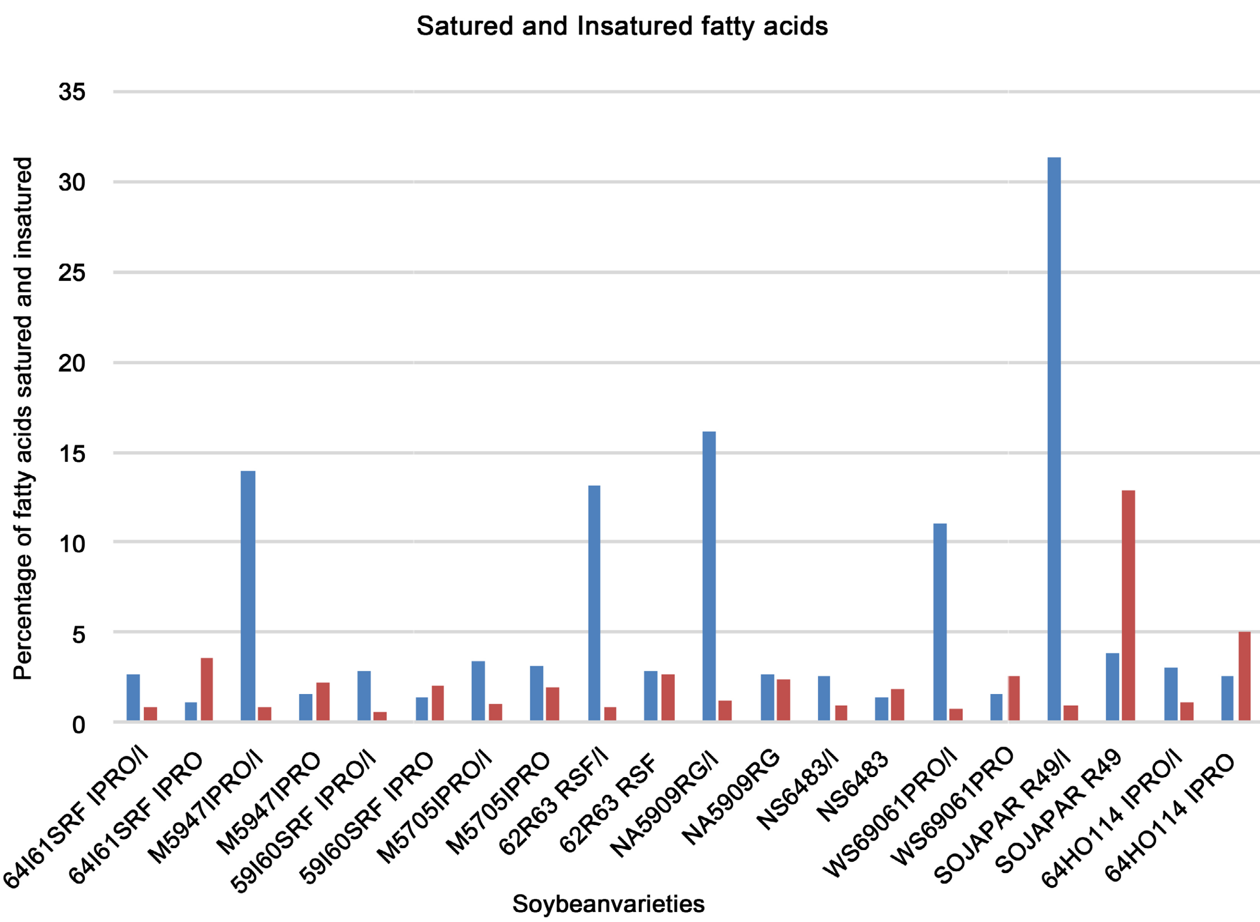

Figure 1. Percentage fatty acids.

reduction in saturated ones compared to the batches of seeds that were not inoculated.

An experiment carried out by [15] in which they evaluated the chemical composition of the oil using seven varieties of soybeans, observed that the degree of unsaturations of the fatty acids influence the chemical composition of the biodiesel and determine its properties, highlighting that the oils with higher proportion of unsaturated fatty acids when converted into biodiesel tend to present low resistance to oxidation.

5. Conclusions

Inoculation with the pathogen *Colletotrichum truncatum* significantly influenced the physiological quality and chemical composition, decreasing both the germination percentage and vigor by 20% to 15% respectively compared to the seed lots that were not inoculated, with differences observed between the evaluated varieties.

This pathogen influenced the chemical composition of the seeds since the inoculated seeds that had alterations in fatty acid levels showed an increase in the levels of saturated fatty acids and a decrease in the levels of unsaturated fatty acids compared to the seed lots that were not inoculated.

Based on the results obtained, the alternative hypothesis proposed is accepted: "The pathogenicity of *Colletotrichum truncatum* influences the physiological quality (germination, vigor, viability) and biochemical components (saturated and unsaturated fatty acids) in different varieties of soybean seeds (*Glycine max*)".

Our results indicate the importance of categorizing soybean varieties as tolerant or susceptible to *C. truncatum*, a proposal for breeders, in addition to promoting this type of research in order to know the damage that this fungus can cause to physiological qualities and chemicals of the final product since the losses can be large and the damage irreversible.

Consent to Publish

This article does not include any clinical studies that require the consent of participants for publication.

The following work is part of Lucas Pérez's dissertation to access the title of Agricultural Engineer, guided by Laura Garay, together they designed the experimental methodology. Laura G wrote the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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