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CHEMICAL AND PHYSICAL EVALUATION OF SOYBEAN SEEDS INFECTED WITH FIVE SOYBEAN MOSAIC VIRUS (SMV) ISOLATES FROM ARGENTINA

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ABSTRACT

Soybean mosaic virus (SMV), causing common mosaic, is the most important soybean virus in Argentina. Recently, five isolated (Marcos Juárez, Manfredi, Venado Tuerto, Vincus Plant and Noroeste Argentino) of SMV were characterized based on their virulence. The aim of this work was to determine and compare the effect of the isolates on selected soybean seed chemical and physical parameters. SMV reduced seed weight and volume. Infection caused a significant increase in protein content but had no effect on oil content of the seeds. Changes in seeds. Changes in seed fatty acid composition were variable; the most important was the decrease of linolenic acid content of SMV infected seeds. Soluble proteins were analysed by SDS-PAGE. There were no differences in protein patterns from infected and uninfected seeds, with exception of the subfraction A_3 (glycinin fraction). Examination of the relation of virus incidence to physical and chemical characteristics of seeds revealed significant positive correlations between incidence and protein content, oleic to linolenic acid ratio and refraction index of the oils. Furthermore there were negative correlations between SMV incidence and stearic and linolenic acid contents, seed weight and volume.

RESUMEN

El virus del mosaico de la soja (SMV), causante del mosaico común, es el más importante en Argentina. Se detectaron diferencias en la severidad de los síntomas ocasionados por el SMV según el área de procedencia de los inóculos, caracterizándose hasta el momento cinco aislamientos (Manfredi, Marcos Juárez, Noroeste Argentino, Venado Tuerto y Planta Vinosa). Los objetivos del trabajo fueron determinar y comparar el efecto de los aislamientos sobre parámetros químicos y físicos del grano de soja. Todos los aislamientos ocasionaron una reducción significativa del peso y el volumen de los granos en relación al testigo sano. La infección produjo un incremento significativo en el contenido de proteínas totales, pero no afectó el porcentaje de aceite de las semillas. Los cambios en la composición de ácidos grasos fueron variables, siendo el más importante la disminución del contenido de ácido linolénico en las semillas infectadas. No se observaron diferencias en los patrones de proteínas solubles entre las semillas infectadas y el control, con excepción de la subfracción A_3 (fracción glicinina). Se determinaron correlaciones positivas entre la incidencia del virus y el contenido proteico, la relación oleico/linolénico y el índice de refracción de los aceites; y negativas con los contenidos de los ácidos esteárico y linolénico, el peso y el volumen de las semillas.

INTRODUCTION

Soybean mosaic virus (SMV) occurs throughout the word [1]. It is the most important xoybean virus in Argentina and it can be found in all the areas of production of the country [2]. Genotype and virus strain are known to play a critical role on soybean mosaic development and yield losses [3-7]. Field inoculation experiments showed that yiel reductions were mostly in the range of 8-35% [3,8,9] but sometimes there were reported to be as high as 94% [10]. At present, the effect of SMV on physical and chemical properties of soybean seeds is not clear. Mbuvi *et al.* [10] reported that SMV infection either had no effect on, or it increased physical parameters such as volume, density or breakage susceptibility, depending on moisture content of seeds.

There is little information about the chemical quality of soybeans infected by SMV. In general, it has been observed that oil content was reduced and protein content was increased by plant inoculation, whereas the fatty acid composition and iodine value of the oil were variable [11-13]. There seems to be no published information about the impact of SMV on soluble seed proteins.

The diversity of environments, cultivars and strains of SMV in Argentina is considerable. Differences in the severity of symptoms caused by SMV according to the inoculum precedence were detected. Thus, it was possible to characterize those of five SMV isolates obtained from different areas of production of Argentina (Laguna, unpublished). However, there has been no investigation to determine the seed quality reduction, if any, fue to the disease. Accordingly, the objectives ot this study were to determine and compare the influence of five different isolates of SMV on some selected physical and chemical characteristics of soybean seeds from Forrest cultivar.

RESULTS AND DISCUSSION

Seed transmission percentage varied significantly among inoculated treatments (Table 1). The mean percentage of transmission of SMV from seeds of inoculated plants among the five isolates, could be separated in two main groups: those close to 73-75% (VT, PV anmd MJ isolates), and those above 90% (NOA and MA isolates). When virus infection was prevented by caging the plants, mottling did not occur and seed transmission percentage was lower than 1%.

TABLE 1. Incidence of different isolates of SMV, and physical properties of seeds from uninoculated (control treatment) and inoculated plants with Manfredi (MA), Vinous Plant (VP), Nororeste Argentino (NOA), Marcos Juárez (MJ), and Venado Tuerto (VT) isolates (inoculates treatments).
Means values ± standard deviation, n =3.

	Control	MA	VP	NOA	MJ	VT
SMV Incidence %	$< 1^{a}$	97.5 ± 0.7^{b}	73.5 ± 0.7^{c}	91.0 ± 1.4^{d}	75.5 ± 0.7^{ce}	73.0 ± 1.4^{c}
Wheght (g/100 seeds)	15.3 ± 0.2^{a}	12.9 ± 0.6^{b}	13.1 ± 0.6^{b}	12.4 ± 0.1^{b}	13.0 ± 0.1^{b}	12.5 ± 0.2^{b}
Volume (cm^3/100 seeds)	13.7 ± 0.5^{a}	11.0 ± 1.4^{bc}	10.1 ± 0.1^{b}	9.9 ± 0.1^{b}	12.1 ± 0.1^{c}	9.9 ± 0.1^{b}

Mean values among treatments having the same letter do not differ significantly at the 0.05 level of probability.

The physical data (Table 1) obtained from SMV infected seeds were unexpected, as compared with those of Mbuvi *et al.* [10]. All the physical parameters measured were lower than those of uninfected seeds. Seeds from plants inoculated with NOA and VT isolates, showed the lowest values of weght and volume. Seed weight and volume presented significant negativa correlations with SMV incidence (Table 2).

TABLE 2. Correlation coefficients between SMV incidence and chemical and physical characteristics of soybean seeds infected with different SMV isolates[a].

Factor	Protein	Stearic	Linolenic	O/Ln	RI	Weight	Volume
SMV	0.5931	-0.8559	-0.6992	0.6108	0.7264	-0.9065	-0.7608

[a] Significant correlations at P=0.05.

TABLE 3. Seed protein and oil contents (g.kg^{-1}, dry basis), fatty acid composition (% of total fatty acids), percentage of unsaturated fatty acids (% US), oleic to linolenic (O/Ln) acid ratios, and iodine values (IV) and refraction indices (RI) or the oils from uninoculated (control) and inoculated soybean plants with Manfredi (MA), Vinous Plant (VP), Noroeste Argentino (NOA), Marcos Juárez (MJ), and Venato Tuerto (VT) isolates of SMV Means values ± standrd deviation, n = 3*.

	Control	MA	VP	NOA	MJ	VT
Protein	270.7 ± 0.1^{a}	290.6 ± 0.9^{b}	342.6 ± 0.9^{c}	360.5 ± 0.3^{d}	349.9 ± 0.6^{cd}	347.5 ± 0.9^{cd}
Oil	227.9 ± 0.2^{a}	236.5 ± 0.1^{a}	256.5 ± 4.2^{a}	223.7 ± 0.4^{a}	220.0 ± 0.5^{a}	233.7 ± 0.7^{a}
Palmitic acid	11.2 ± 0.3^{a}	11.9 ± 0.8^{a}	12.0 ± 0.2^{ab}	12.2 ± 0.1^{ab}	9.1 ± 2.8^{ac}	11.9 ± 0.1^{a}
Stearic acid	3.4 ± 0.1^{a}	2.9 ± 0.1^{b}	3.1 ± 0.1^{b}	3.1 ± 0.1^{b}	3.1 ± 0.1^{b}	3.0 ± 0.1^{b}
Oleic acid	18.5 ± 0.3^{a}	20.1 ± 0.5^{b}	17.9 ± 0.5^{a}	20.6 ± 0.3^{b}	18.1 ± 0.1^{a}	20.5 ± 01^{b}
Linoleic acid	58.3 ± 0.2^{a}	59.1 ± 1.2^{a}	59.5 ± 0.3^{a}	58.1 ± 0.2^{ab}	61.4 ± 2.0^{ac}	58.6 ± 0.8^{a}
Linoleci acid	8.6 ± 0.3^{a}	5.9 ± 0.3^{b}	7.4 ± 0.1^{c}	6.0 ± 0.2^{b}	8.3 ± 0.1^{ac}	5.9 ± 0.8^{b}
% US	85.4 ± 0.2^{a}	85.1 ± 0.9^{a}	84.7 ± 0.3^{ab}	84.7 ± 0.3^{ab}	87.8 ± 2.7^{ac}	85.03 ± 0.1^{a}
O/Ln	2.2 ± 0.1^{a}	3.4 ± 0.2^{b}	2.4 ± 0.1^{a}	3.4 ± 0.1^{b}	2.2 ± 0.1^{a}	3.5 ± 0.5^{b}
IV	145.9 ± 0.9^{ab}	141.4 ± 2.4^{a}	144.4 ± 0.4^{a}	140.4 ± 0.4^{a}	150.4 ± 4.9^{b}	140.9 ± 0.8^{a}
RI	1.449 ± 0.1^{a}	1.466 ± 0.004^{b}	1.468 ± 0.003^{b}	1.463 ± 0.001bc	1.474 ± 0.001bd	1.472 ± 0.002^{b}

* Mean values among treatments having same letter do not differ significantly at the 0.05 level of probability.

Regarding the seed chemical composition (Table 3), it was noteworthy the increase in protein content in SMV infected seeds, specially those from PV, VT, MJ and NOA isolates. There was a positive correlation between protein content and SMV incidence. In the other hand, oil content did not present statistically significant differences between treatments. These results agree partially with those of Demski and Jellum [14], and Suteri [11] who found that SMV infection caused an increase in protein content and a decrease in oil content of soybean seed.

All fatty acids analysed varied significantly among treatments. In general, SMV infection decreased the amounts of linolenic acids, while changes in palmitic, oleic and linoleic acids were variable. The stearic acid content did not differ significantly among isolates, but it was significantly different from that of the control. Comparing the control to the SMV inoculated treatments, the greatest changes were ad follow: palmitic acid showed a 19% decrease in MJ isolate, stearic acid a 15%

decrease in Manfredi isolate, oleic acid an 11% increase in NOA isolate, linoleic acid a 5% increase in MJ isolate, and linolenic acid a 31% decrease in Manfredi isolate.

SMV seed infection reduced in the total percentage of unsaturated fatty acids and iodine values with exception of seeds from MJ isolate; however, it increased the refraction indexes of the oils. An interesting feature was the oleic to linolenic acid (O/Ln) ratio increase in seed oils from inoculated plants: a positive correlation between seed transmission percentage and O/Ln ratio was found at r = 0.6108, whereas linolenic acid content correlated negatively with seed transmission percentage (Table 2).

Most of the proteins examined by SDS-PAGE were storage proteins, which are the major constituents of seed proteins [15]. The patterns produced from extracts of different seed samples were compared visually on the basis of differences in presence/absence of specific bands. Since we had used saline buffer solution for extraction of seed proteins, the majority of the bands were likely due to globulins. The 7S (ß-conglycinin) and 11S (glycinin) globulins constitute the main storage proteins of soybean [16]. The three individual subunits (a, a' and ß) of the ß-conglycinin did not differ between infected and uninfected (Fig. 1). Regarding the composition of the 11S fraction, degradation of the 11S fraction, degradation of the subfraction A_3 was detected in seeds infected with the different SMV isolates.

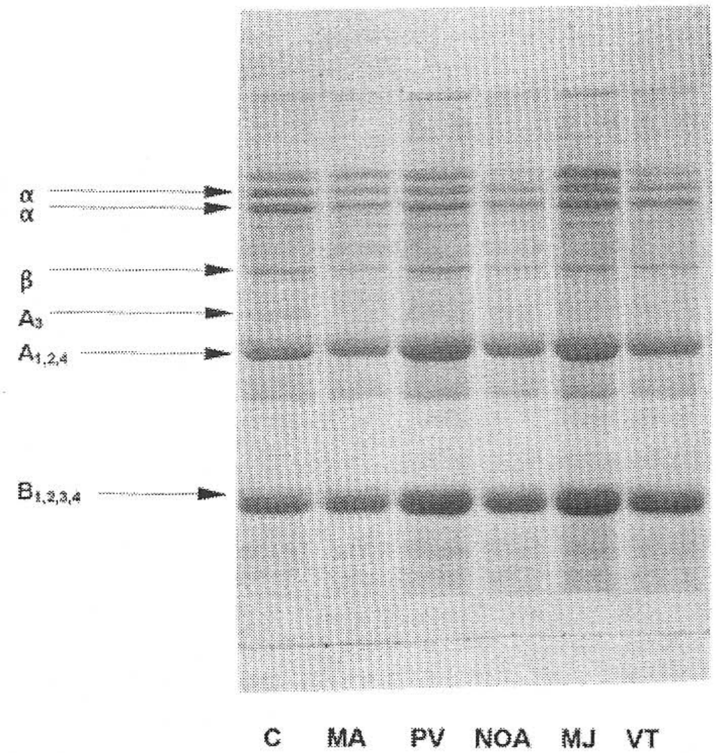

FIGURA 1. Electrophoretic separation of soybean seed extracts in SDS-gels. Bands labeled α', α and ß are subunits of ß-conglicin, bands A_n y B_n are acidic and basic polypeptides, respectively, of glycinin.
Control treatment: C. Inoculated treatments: Manfredi, MA; Vinous Plant, PV; Noroeste Argentino, NOA; Marcos Juárez, MJ and Venado Tuerto, VT.

In summary, the data obtained in this work and other related [10,12,14] allow to draw the following conclusions: a) SMV caused significant reduction of seed weight and volume; b) although SMV inoculation increased significantly the protein content, it had no effect on the oil content of the seeds; c) in spite of the high percentage of SMV incidence in inoculated treatments, the oil quality was not damaged, as the percentages of individual fatty acids, iodine values and refraction indices were still well within the normal range of edible seed oils; d) finally, SMV infection practically did not affect the seed protein quality. This is very important because structural and quantitative variations in storage proteins, like globulins influence both nutritional quality and functional properties of soybean foods [16,17].

MATERIALS AND METHODS

Plant materials. Isolates of SMV were obtained from different areas of production of production of Argentina: Marcos Juarez (MJ) and Manfredi (MA) (Córdoba province), Venado Tuerto (VT) and Vinous plant (VP) (Santa Fe province), and Noroeste Argentino (NOA) (Salta and Tucumán provinces).

Plants from Forrest cultivar at first trifoliate stage (V2) were inoculated separately with one of five isolates of SMV (inoculated treatments) and then kept under greenhouse (22°C ± 2° C) conditions until production of seeds (R8). Control plants (uninoculated treatment) were grown under cages to prevent virus infection by excluding insect vectors.

Three replicates from each treatment were used. Seeds of each treatment/replicate combination were harvested separately by hand at maturity. Fitty seeds were soaked, then the seed coats were separated with a scalpel and tested for the presence of SMV using the DAS-ELISA serological test [13].

Physical analyses. Samples of 50 seeds were randomly selected from each treatment / replicate combination. Each samples was weighed by using a Mettier analytical balance and its volume was calculated according to Hepperly and Sinclair [18],

Chemical analyses. *Moisture, protein and oil contents.* Moisture content was determined using the oven drying method [19]. Protein and oil contents were determined according to the methods of the AOAC [20].

Fatty acid analysis. The crude oils were subjected to alkaline saponification (1N KOH in methanol). Unsaponifiable matter was extracted with *n*-hexane. The fatty acid methyl esters (FAME) of total lipids were obtained using 1N H_2SO_4 in methanol and analysed by gas chromatography (GC) according to Maestri et al. [21]. Iodine values (IVs) of oils were calculated from fatty acid percentage [22] by means of the formula IV = (% oleic x 0.899) + (% linoleic x 1.814) + (% linolenic x 2.737).

Soluble protein analysis. Soluble proteins were extracted by the method of Zimmerman and Vick [23]. The extracted proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) using 1-D vertical salb gel containing 3% stacking gel and 10% separating gel. Por comparison, proteins of known molecular weight e.g. bovine serum albumin (M 66.000), pepsin (M 34.700), trypsinogen (M 24.000) and lysozyme (M 14.300) were subjected to electrophoresis under identical conditions. Proteins were also identified by bibliography [16].

Statistical analysis. Statistical differences between treatments were estimated from one-way ANOVA test at the 5% level (P = 0.05) of significance. Least significant difference (LSD) was performed to establish relationships between treatments. Correlation analysis was carried out employing Pearson's test [24].

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