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Physiological Seed Quality and Fungi Incidence in Wheat (*Triticum aestivum* L.) Seeds Treated with Microwave Radiation

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Abstract

The effects of microwave oven on fungi seed disease control and its effects on physiological seed quality were evaluated in four wheat varieties ('Tlaxcala', Batán, 'Rebeca', and 'Triunfo'). Seeds were irradiated by microwave oven over 10, 20, and 30 seconds. A randomized experimental design with four repetitions of 25 seeds each was used. Fungi presence in seeds was evaluate using potato dextrose agar (PDA) media, while physiological seed quality was tested using a standard germination test. The lower fungi incidence was associated to 30 seconds of radiation; this treatment also showed good seed germination (73.75%), as well as low percentage of abnormal seedlings (13.50%), and non-germinated seeds (12.75%), respectively. Radiation for 30 seconds reduced seed fungi contamination by *Penicillium* and *Alternaria.* 'Rebeca' variety showed the highest seed quality, therefore suggesting that the genotype could have an important role. Microwave oven treatment of wheat seeds could be a useful method to significantly reduce fungi presence on seeds.

Keywords: germination, vigor, seed disease, cereals, seed quality

Introduction

In Mexico, wheat presents diseases caused by diverse biological agents such as bacteria, fungi, nematodes, and virus, which are transmitted from one plant to another one, sometimes through seeds, which cause problems to both, researchers and producers, since not only cause yield reduction, but also cause dissemination of the disease to new locations. Several methods have been tested to eliminate seed fungi: chemicals, physical and biological ones; however, these methods must be crop specific, otherwise seed germination and seed vigor could be affected negatively (Reddy *et al*., 2000).

Microwave oven radiation is a temperature treatment that has been used for several years to kill microorganisms in small seed volumes. This is a promising technique since an increase in temperature eradicates fungi, particularity in their mycelium stage, because spores are more resistant. Cavalante and Mochovej (1993) consigned that Watson, Coltrin and Robinson reported for the first time the use of microwave in 1951, to eliminate internal fungi in legume seeds before storage. Microwave treatment has been recently reused by researchers to kill fungi in soybean seeds (Reddy *et al*., 2000).

Microwave treatment has been used to eliminate soil pathogens such as *Phytium*, *Fusarium*, and several nematodes, with the exception of *Rhizoctonia*. Treatment effect of microwave radiation decreases when the quantity of irradiated soil is low and it increases when water content of soil samples increases (Ferris, 1984). When wheat seeds infested with *Fusarium graminearum* were irradiated, seed quality reduced by 4 to 7 %, while seed germination kept at 85 %. When microwave radiation is increased, it could kill the pathogen but also it decreases seed viability and germination, therefore, it was concluded that optimal irradiation time was 20 seconds (Bhaskara *et al.*, 1998). In bean, microwave radiation could stimulate seed germination if temperature is lower than 50 ^{o}C (Spilde, 1987). Therefore, the main objectives of in this study were to evaluate the effects of microwave radiation on wheat seed fungi control and the physiological seed quality.

Materials and Methods

Seeds of four wheat varieties: 'Batán', 'Rebeca', 'Triunfo', and 'Tlaxcala', were previously classified by size using oblongs screens No. A 12/64” Seed Buro®. Afterwards, they were exposed for 0, 10, 20, and 30 seconds in a microwave Panasonic® NN-N740 (120V, 60 Hz, 120 Amps, 1200 W). Twenty five seeds of each variety were placed into test tubes and then placed into the microwave oven together with 150 ml of water into a 250 mL beaker in order to reduce heat damage to seed coats (Reddy *et al*., 2000). Fresh water was sited into the oven each time a sample was introduced in order to avoid an increase in its temperature due to continuous exposition. In addition, at the end of each treatment period the seed temperature was measured using a Broker Thermometers® 260 ^{o}C thermometer.

After treatments, standard seed germination was conducted using a paper roll test under a complete randomized experimental design, using four repetitions of 25 seeds each. For fungi evaluation, seed samples were manipulated under laminar flux chambers. In order to eliminate external fungi on seed covers due to management, they were placed into timsen solution. Afterwards, they were placed into Potato Dextrose Agar (PDA) medium in Petri dishes, previously sterilized laying 25 half seeds in each box. There were four repetitions for each treatment.

Physiological seed quality. Eight days after sowing, the following traits were evaluated: percentage of normal seedlings (PNS), percentage of abnormal seedlings (PAS), and non-germinated seeds (NGS). In addition, a 10 seedlings sample per variety was used to measure root length (from the root tip to seedling neck), and shoot seedling length (from seedling neck to last leave tip). Finally, the seedlings were dried and dry weight was determinate.

Fungi presence. Five days after sowing, percentage of infected seeds was estimated. Pathogen identification was done using Warham *et al*. 1994's Manual.

Statistical analysis. Results were subjected to an analysis of variance, multiple means Tukey's test, and Person's correlation analysis ($\alpha = 0.01$) using SPSS software.

Results and Discussion

Significant differences were found for varieties and time of radiation for most evaluated traits, except for seedling dried weight (PDW) and seed temperature (ST), where there were no significant differences among varieties (Table 1). These results could be originated from distinct environmental conditions during seed development, plant nutrition, physical damage, or seed ageing among other factors (Thomson, 1983; McDonald, 1985).

Table 1. *Mean Squares of Physiological Seed Quality and Fungi Presence in Four Wheat Varieties Seeds Treated by Microwave Oven Radiation*

SV	DF	PNS	PAS	NGS	SL	RL	PDW	F	ST
Var	3	2534.0**	628.2**	733.5**	45.1**	36.9**	0.1	2175.1**	1.8
Tre	3	1080.6**	222.9**	332.2**	14.0**	46.5**	0.02	12781.5**	707.5**
Var*Tre	9	42.6	11.8	32.4	8.4	4.10	0.03	78.50	2.71
Error	48	99.83	47.21	104.04	4.23	3.66	0.06	58.50	6.28
Total	64								
Mean		64.75	17.06	18.19	11.50	11.24	0.20	52.46	29.25
C. V.		0.25	0.52	0.64	0.23	0.24	1.16	0.43	0.21

SV: Source of Variation; DF: Degrees of Freedom; PNS: Percentage of Normal Seedlings; PAS: Percentage of Abnormal Seedlings; NGS: Percentage of Non-Germinated Seeds; SL: Shoot Seedling Length; RL: Root Length; PDW: Seedling Dried Weight; F: Fungi Percentage; ST: Seed Temperature; Var: Variety; Tre: Exposition Time into Microwave; CV: Coefficient of Variation; **: Significant ($P<0.05$); NS: No Significant.

Coefficients of variation were lower than 1 % for most of evaluated traits, with the exception of dried weight, therefore obtained results could be considered reliable. Non-significant interaction between the two factors under study was detected.

Variety effects. Means comparisons test results showed that 'Rebeca' variety presented the highest percentage of normal seedlings (80.75 %), which was under permissible quality standards of seed certification (Table 2). In addition, percentage of abnormal seedlings and non-germinated seeds were 8.5 % and 10.7 %, respectively. This wheat variety also showed the longest seedlings, both shoot and root; however, there were no statistical differences among varieties in seedling dried weight, probably due to initial seed classification by size and therefore the same amount of seed reserves, and therefore not allowing finding variety differences, since they are directly responsible of seedling weight (Copeland and McDonald, 2001).

Table 2. *Means Comparisons of Physiological Seed Quality Traits and Fungi Presence in Four Wheat Varieties*

Variety	PNS (%)	PAS (%)	NGS (%)	SL (cm)	RL (cm)	F (%)
ʻTlaxcalaʼ	56.00c[z]	22.75a	21.25ab	11.05b	10.10bc	56.25a
ʻBatánʼ	53.50c	20.50a	26.00a	10.14b	9.45c	58.13a
ʻRebecaʼ	80.75a	8.50b	10.75c	13.95a	13.15a	43.00b
ʻTriunfoʼ	68.75b	16.50a	14.75bc	10.87b	11.35ab	2.00c
HSD	13.66	12.61	3.28	1.76	1.59	2.34

[z]Means with the Same Letter in Columns are Statistically Equals (Tukey, α=0.05); PNS: Percentage of Normal Seedlings; PAS: Percentage of Abnormal Seedlings; NGS: Percentage of Non-germinated Seeds; SL: Shoot Seedling Length; RL: Root Length; PDW: Seedling Dried Weight; F: Fungi Percentage; ST: Seed Temperature; HSD: Honestly Significant Difference.

'Batán' and 'Tlaxcala' varieties showed the lowest seed physiological quality and the highest fungi infestation, therefore suggesting that 'Rebeca' and 'Triunfo' were less susceptible to fungi infection. Because all wheat varieties were growth and managed under the same conditions, recorded difference could be attributed to genetic factors, since vigor and stress resistance depend on genotype (Thomson, 1979).

Radiation exposition. Highest seed temperature (37.2 ^{o} C) was obtained when exposed for 30 seconds to microwave radiation (Table 3), and consequently fungi incidence was reduced 75 %; in addition, physiological seed quality also increased, since percentage of germination and root length increased 34 % and 44%, respectively. These results could had been obtained as a consequence of a favorable pre-conditioned temperature effect on metabolic mechanisms involved in seed germination and emergence (Bhaskara *et al*., 1998), effects that had been previously recorded for bean (Spilde, 1987). Radiation for 10 and 20 seconds also reduced fungi incidence without undesirable effects on seed physiological quality; these results agreed to those

obtained by Bhaskara *et al*. (1998) who concluded that the best radiation treatment was between 20 and 30 seconds of exposition.

Table 3. *Means Comparisons of Physiological Seed Quality and Fungi Presence on Wheat Treated by Three Levels of Microwave Oven Radiation*

Time	PNS (%)	PAS (%)	NGS (%)	SL (cm)	RL (cm)	F (%)	ST (°C)
0	55.00c[z]	21.50a	23.50a	10.37a	9.13c	79.83a	22.00d
10	61.50bc	18.75ab	19.75ab	11.08a	10.86bc	59.83b	26.06c
20	68.75ab	14.50 b	16.75ab	12.27a	11.78ab	44.83c	31.69b
30	73.75a	13.50 b	12.75 b	12.28a	13.20a	1.00 d	37.25a
HSD	13.66	5.55	3.82	1.76	1.59	2.34	4.00

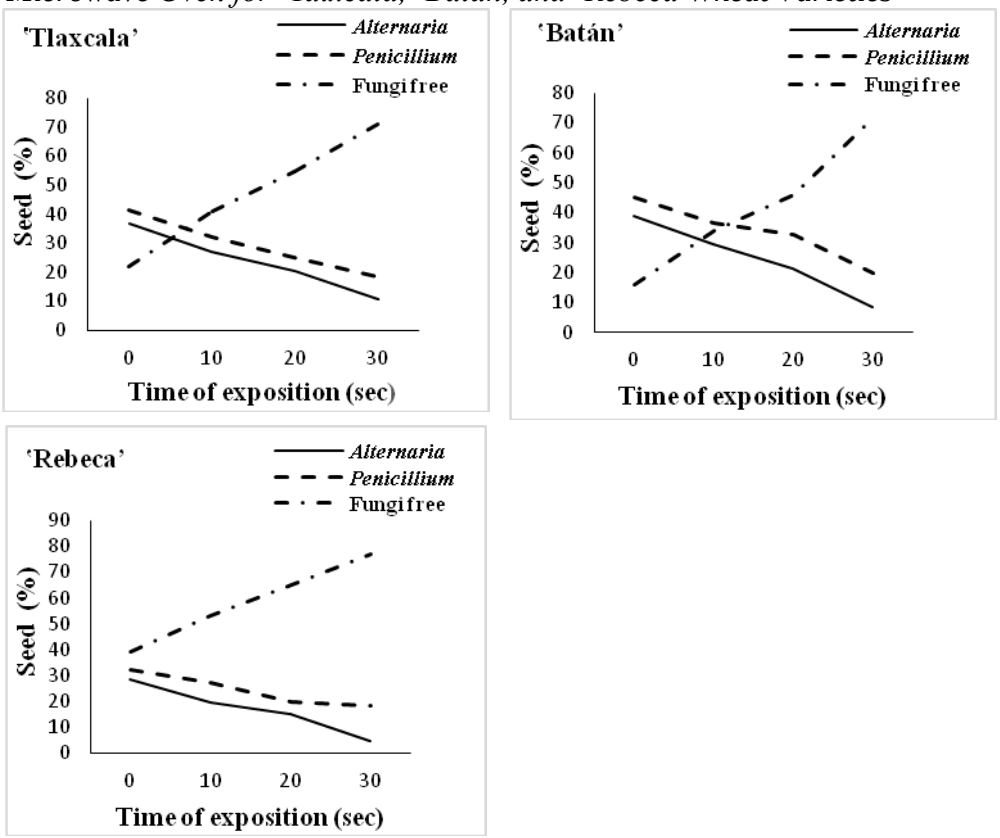
[z]Means with the Same Letter in Columns are Statistically Equals (Tukey, α=0.05); PNS: Percentage of Normal Seedlings; PAS: Percentage of Abnormal Seedlings; NGS: Percentage of Non-germinated Seeds; SL: Shoot Seedling Length; RL: Root Length; PDW: Seedling Dried Weight; F: Fungi Percentage; ST: Seed Temperature; HSD: Honestly Significant Difference.

All treatments evaluated in this study were favorable because they did not reach 40^{o}C, when denaturation of proteins starts (Delouche, 1982), and therefore germination and vigor is reduced drastically (Reddy *et al*., 2000), or at least seed coats are affected (Cavalante and Muchovej, 1993; Reddy *et al*., 2000).

The highest percentage of healthy seed was observed for 30 seconds of exposition in the microwave oven, since incidence of *Alternaria* and *Penicillium Link Nees* was reduced when temperature increased (Figure 1). This behavior has been observed before for wheat seeds, since increases in temperature reduced *Fusarium graminearum* incidence (Bhaskara *et al*., 1998).

Microwave oven is a promising tool since when temperature increases fungi are eradicated, particularly when they area at their mycelium form, since spores are more resistant (Cavalante and Muchovej, 1993). Previous studies on the utilization of microwave had not been concluding; however it had be demonstrated that its use can reduce wet soil microorganism's infestation (Ferris, 1984). In addition, microwave radiation can stimulates germination if seed temperature does not increase up to 50 ^{o}C (Spilde, 1987).

Figure 1. *Evaluation of Fungi Percentage Related to Exposition Time in a Microwave Oven for 'Talxcala, 'Batán' and 'Rebeca' Wheat Varieties*



Correlation analysis. Results from correlation analysis showed a positive effect on vigor expression (seedling and root length) and germination (percentage of normal seedlings) as a result of the increase of seed temperature (ST), as well as a negative effect on percentage of abnormal seedlings (PAS) and fungi incidence (F). These results support those previously presented in the mean comparisons tests. There is no significant relationship between fungi incidence and seed physiological quality; however there is a positive and significant correlation between percentage of germination (PNS) and seedling length (SL and RL).

Table 4. *Correlation Coefficients for Physiological Seed Quality Traits, Fungi Presence, and Seed Temperature on Wheat Seeds*

	PNS	PAS	NGS	SL	RL	PDW	ST	F
PNS	1.0	-0.70**	-0.84**	0.54**	0.64**	-0.05	0.45**	-0.02
PAS		1.0	0.20	0.34**	-0.43**	0.17	-0.39**	-0.11
NGS			1.0	-0.48**	-0.55**	-0.06	-0.32*	0.11
SL				1.0	0.68**	0.06	0.28*	0.05
RL					1.0	0.13	0.48**	0.13
PDW						1.0	0.09	0.25
ST							1.0	-0.14*
F								1.0

PNS: Percentage of Normal Seedlings; PAS: Percentage of Abnormal Seedlings; NGS: Percentage of Non-germinated Seed; SL: Shoot Seedling Length; RL: Root Length; PDW: Seedling Dry Weight; ST: Seed Temperature; F: Seeds with Fungi; **: Highly Significant (α=0.01); *Significant (α=0.05).

Conclusions

Use of microwave oven can reduce fungi infestation in wheat seed without negative effects in seed germination and vigor.

Seeds exposed for 30 seconds to microwave radiation showed less fungi infestation not affecting seedlings physiological quality.

Low fungi infestation levels on 'Rebeca' wheat variety beneficed physiological seed quality expression; therefore, showing that genetics could be determinant.

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