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Original article

Interaction of Seedling-Pathogens with Physiological Seed Quality Affecting Soybean Emergence and Seedling Growth

Sema Şahbaz [a, *], Gary Munkvold [b], Silvina Arias [b] & Fatih Mehmet Tok [a]

[a] Department of Plant Protection, Faculty of Agriculture, University of Mustafa Kemal, Hatay, Türkiye
[b] Iowa State University, Dept. Plant Pathology & Microbiology- Seed Science Center, Ames, IA, USA

Abstract

Seed vigor is a complex trait which refers the quick and uniform germination of seeds in the field. It can be highly affected by the genetic background of the seed, the environment where the seeds are grown, and storage conditions (Yang, X. B. 1999). Besides, seeds affected by quality parameters may respond differently to seedling pathogens in the soil; these responses are likely to differ according to environmental conditions. The main objective of the study was to evaluate the relationship between soybean quality and the effects of specific soil-borne pathogens on soybean emergence and seedling growth, with a specific focus on phenotyping early-stage roots. Seed lots with different levels of seed quality, represents a range of seed vigor with the same genetic background, were created by accelerated-aging (aa) treatments. The effect of aa on seed performance was tested in growth chambers with and without infested soil at 20 °C and 25 °C. A remarkable emergence reduction (65-55%) was observed in the aged-seed. Synergistic effects between seed aging and *Rhizoctonia solani* infestation was observed on root biomass (root dry weight) and the numbers of root tips, forks, and crossings ($p<0.05$). Besides, some parameters such as plant length and fresh weight, fresh root weight, root length, volume, and surface area were significantly affected by both seed quality and fungal inoculum. The results obtained from the study is expected to contribute on determining the impact of environmental conditions and stress factors on the epidemiology of soilborne pathogens. On the other hand, we expect that the results will shed light on developing new strategies for effective disease management.

Keywords: Soybean, Soil-born, Rhizoctonia, Seed quality, Seed vigor.

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* **Corresponding author:**

Sahbaz, S. is an Researh Associate in Corteva Agriscience in Adana, Türkiye. Her research interests include the Plant Pathology, Plant Breeder, Agronomy, She has lived, worked, and studied in Adana, Türkiye.
Email: semasahbaz@gmail.com

INTRODUCTION

Soybean (Glycine max. Merr.) is an annual summer crop from the *Leguminosae* family. Its seeds contain 36-40% protein, 18-24% oil, 26% carbohydrate and 18% mineral substances. Due to these valuable nutrients, it is known as the "wonder plant" of the century (Arıoğlu, 2007). Soybean is one of the oldest plant species cultivated by mankind. The origin of the soybean is known as Eastern Asia. The first cultivation of the soybean in Turkey was during World War I. It has been planted as main crop in Black Sea region. Later on, it was planted as second crop with new released varieties in the Mediterranean and Aegean regions after the year 1968 (İlisulu 1983; Deniz 1988). As a summer and short-day plant, the optimum temperature for germination is 15 °C while 25-30 °C for photosynthetic activities (Saglam timur et al., 1998). The soil composition which is rich for organic matter and sandy-loamy soil type may increase the yield for soybean crops. Besides, optimum pH is range between 6.2-6.8 for soybean growth and development. Molybdenum uptake which is necessary for nodulasation decrease at lower pH levels while uptake of other elements such as iron, copper and zinc decrease at higher pH levels (Arıoğlu, 1999).

Rhizoctonia solani causes seedling blight, including pre-emergence and post-emergence damping-off and root rot in young and adult soybean plants (Xue et al. 2007; Zhang et al. 2013; Chang et al. 2015b; McLaren et al. 2015). In the United States, rhizoctonia root and hypocotyl rot causes lesions and damping-off on the hypocotyl and roots of soybean (Wrather 2001). In Ontario, Canada, rhizoctonia root rot ranked fourth among 22 diseases causing losses in soybean between 1994 and 2000 (Anderson and Tenuta 2001).

Rhizoctonia root rot of soybean caused yield losses of up to 45% in the United States (Muyolo et al. 1993). The Rhizoctonia and pythium root rot complex caused an estimated 108,000 tons of yield loss in soybean in Brazil, Canada, Indonesia, and the United States (Wrather et al. 1997). In Brazil, *R. solani* caused pre-emergence and post-emergence damping-off, root and hypocotyl rot and leaf blight in soybean, resulting in an estimated 31%-60% yield loss (Fenille et al. 2002).

Rhizoctonia solani can cause pre- and postemergence damping off, root rot, and hypocotyl lesions on soybeans in the United States as well as web blight in the southern United States . Seedling diseases caused by *R. solani*, Pythium spp., and/or Fusarium spp. ranked fourth overall among diseases causing losses on soybeans in the United States during 1996 to 1998 (Wrather, J. A., Stienstra, W. C., and Koenning, S. R. 2001) . *R. solani* along with Pythium spp. and *Phytophthora sojae* were identified as the major causal agents in soybean seedling disease in Iowa (Rizvi, S. S. A., and Yang, X. B. 1996. Fungi associated with soybean seedling disease in Iowa. Plant Dis. 80:57-60). Epidemics of *R. solani* damping-off have occurred in soybeans, with reported yield losses up to 50% of total production. *R. solani* is divided into anastomosis groups (AG) based on hyphal anastomosis and cultural characteristics .

Deterioration of soybean seed is faster than cereals crop seed due to high protein and fat contents (Lisjak et al., 2009). Sofalian et al. (2015) noticed that polymorphism of storage protein on seed of soybean genotypes could be used as for selection. Soybean seed viability among cultivars showed gradual decreasing with increasing storage periods up to six months (El-Abady et al., 2012). Mbofung et al. (2013) noted that soybean seed viability expressed by germination percentage was affected by environmental factors during storage.

The Accelerated Aging (AA) test that established an injurious environmental condition (high temperature and relative humidity) for a specific period is applied in the chamber to evaluate the storability of seed lots (Gupta, 1993). The effectivity and good accuracy of accelerated aging with high temperature and RH for minimum 24 hours had been noted to predict relative storability and field emergence of soybean (TeKrony and Egli, 1997; Torres et al., 2004; Shivasharan Appa et al., 2017). Demir and Mavi (2007) also noted the utilization of accelerated aging on melon seed lots for predicting seedling emergence.

Seed vigor is a complex trait that indicates the capacity of the seed for germination and field emergence; vigor can be impacted by genetic background, the environment where the seeds are grown, and storage conditions (Finch-Savage and Bassel, 2016; Sun et al, 2007). In addition, seeds affected by quality parameters may respond differently to seedling pathogens in the soil; these responses are likely to differ according to environmental conditions..

The goal of this study is to evaluate the relationship between soybean quality and the effects of specific soil-borne pathogens on soybean emergence and seedling growth, with a special focus on phenotyping early-stage roots.

MATERIALS AND METHODS

Inoculum Preparation

R. solani isolates used in these studies are listed in Table 1. *R. solani* colonized oats were produced by first soaking 600 ml of oats with 500 ml of water in 2.8-liter Erlenmeyer flasks overnight. Then, millets were autoclaved for 1 h each on two consecutive days in the flasks. 3-5 plugs of *R. solani* (5 mm in diameter) were cut and added to each flask from a 5 to 7 day-old colony on water agar. The flasks were shaken daily for 3 weeks to ensure colonization until the colonies on the millets were reached to approximately 25 mm in diameter. Inoculum was placed on brown paper to air dry when the millets were well colonized. The inoculum was mixed and seperated into individual kernels daily, placed in plastic bags and stored at 4°C until used. Control treatments or non-inoculated did not have any millets added to the pots.(Sneh, B., Burpee, L., and Ogoshi, A. 1991)

Temperature Effects

Two growth chambers, with temperatures set at 20°C and 25°C, were used to evaluate the effects of temperature on *R. solani* disease development. *R. solani* isolate and a non-inoculated control were used in this experiment. The soybean seed and inoculum were covered with approximately 2.5 cm of vermiculite and placed in the growth chambers at the set temperatures and light/dark cycle of 12 h. Pots were watered twice daily.

Soil Infestation

Millet seeds were soaked in water for 24 h, drained and autoclaved at 121˚C for 1 h in autoclave bags with a micro-porous filter patch on two consecutive days. Isolate was grown on potato dextrose agar (PDA) 39g/L (Difco, Becton, Dickinson and Co, Spark, MD,USA) for 14 days in an incubator at 25˚C with a 12 h photoperiod, in order to promoteconidial formation. Plugs (~1 cm2) from cultures of *R.solani* were added to the sterile millet in abiosafety cabinet, and then sealed with a rubber band. Bags were then placed in an incubator (Hoffman manufacturing Inc. Oregon, USA) for 8 days, at 65% relative humidity with a 12 h photoperiod, and mixed by hand every day. Fg-infested millet was mixed with sieved soil at 10% concentration by volume. Infested-autoclaved millet was mixed with sieved soil served as control treatments (Kleinhenz, M. D., and Palta, J. P. 2002) *Rs*-infested millet was mixed with field soil at 1% concentration by volume. Autoclaved sterile millet mixed with soil was used as non-infested control treatments.

Figure 1. Autoclaved- millet

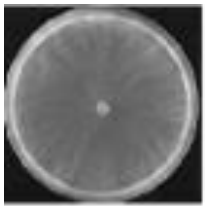

Figure 2. *R. solani* Plugs (1 cm2)

Accelerated Aging (AA) Treatment

To evaluate the relation between low seed quality and soilborne seedling pathogens, we conducted preliminary accelerated-aging treatments and standard soybean germination to determine the protocol to use for pathogen inoculation experiments.

The purpose of the accelerating aging treatment is to reduce the germination rate of the seeds, which will use for pathogen inoculation experiments later (Sun, Q., J. Wang, and B. Sun. 2007).

The aging period ranges from 48 to 96 hours as depending on type of seed (corn and soybeans: 72 hours). Losses in seedling quality are expected in the interaction of poor seed quality with Rhizoctonia solani, a soil-borne disease. The seeds used in the study were kept in an aging cabinet for 3 days. In this experiment, 72 hours was taken into consideration since germination was low in seeds waiting more than 3 days.

Figure 3. Soybean seed for AA test

Table 1. Aging treatment and germination (%)

Treatment	Temp (°C)	Age period (h)	Germination (%)
AA Normal	41	48	97
AA Modified	41	72	94
AA Modified	41	96	88

Soybean Seeds

High- and low-quality soybean seedlings with a range of seed vigor with same genetic backround, which are obtained via accelerated-aging (aa) treatments, were used as material in the study. The effect of aa on seed performance was tested in growth chambers with and without infested soil at 20°C and 25°C (Sun, Q., J. Wang, and B. Sun. 2007) Root morphological measurements were analyzed

by WhinRHIZO root-scanning software. Statistical analysis was carried out by JM P statistical analysis package program.

Planting at 20 & 25 ℃

Two separate germination cabinets at 20 C and 25 C were used in the experiment to observe root growth. the experiment was set up when the germination chambers reached the appropriate temperature conditions.

The soil for sowing the seed was mixed with infected white millet (1%) for artificial inoculation while the non-infected white millet was mixed with the soil for the control treatment. The infected soil was sown with both the germination-tested seed. A control seed was planted as a control. In the other pot, the aging-tested seed and control seeds were sown in the non-infected soil.

Figure 4. Planting to Soybean (for agency seed/control seed)

Harvest and Data Analysis

Plant height was considered as harvesting maturity. When the plant height of control plants reached the equal length and homogenous appearance, Harvesting is carried out at the end of fourth week after sowing. Data were collected after 3 weeks on the number of plants that emerged, number of plants with lesions, average plant height, top fresh weight, and root rot rating. The root rot rating scale was as follows: 1 = no root rot; 2 = 1 to 33% of roots with visible lesions or root rot; 3 = approximately 33 to 50% of the roots rotted or damaged; 4 = 50 to 80% of the roots rotted; and 5 = preemergence damping-off and few if any roots.

Harvesting was done by removing the soil from the roots of the plants without damaging the plant roots and stems. All roots were sorted separately (Figure 5).

Figure 5. Harvest time for trails and harvested root image

Root rot was visually graded for severity (%). Roots were scanned using a flatbed scanner (EPSON) and root images were analyzed using WinRhizo software 2008. Data on root length, surface area, root volume, root tips, forks were taken from the roots.

Shoot and root dry weights were measured on each individual plant after oven drying at 80˚C for 24 h. One pot was considered as an experimental unit with 10 seeds or subsamples in each pot. The experiment had 10 replications (Bolkan, H. A., and Ribeiro, W. R. C. 1985)

RESULTS AND DISCUSSION

Root Quality

The main effect of temperature was significant for root rot rating for the isolates evaluated in this study. There was a significant difference in the number of hypocotyl lesions that developed on the plants across the temperatures (Figure6/7). Low-quality seeds and R. solani affected negatively the root morphology characteristics at 20 ℃. Statistical difference for the root quality was not observed in the interaction between seed quality - inoculum (SxI). There were few significant effects at 25℃ .

Figure 6. Effect of *R. solani* on soybean seedling from aged-seeds (above) and good quality seeds (below).

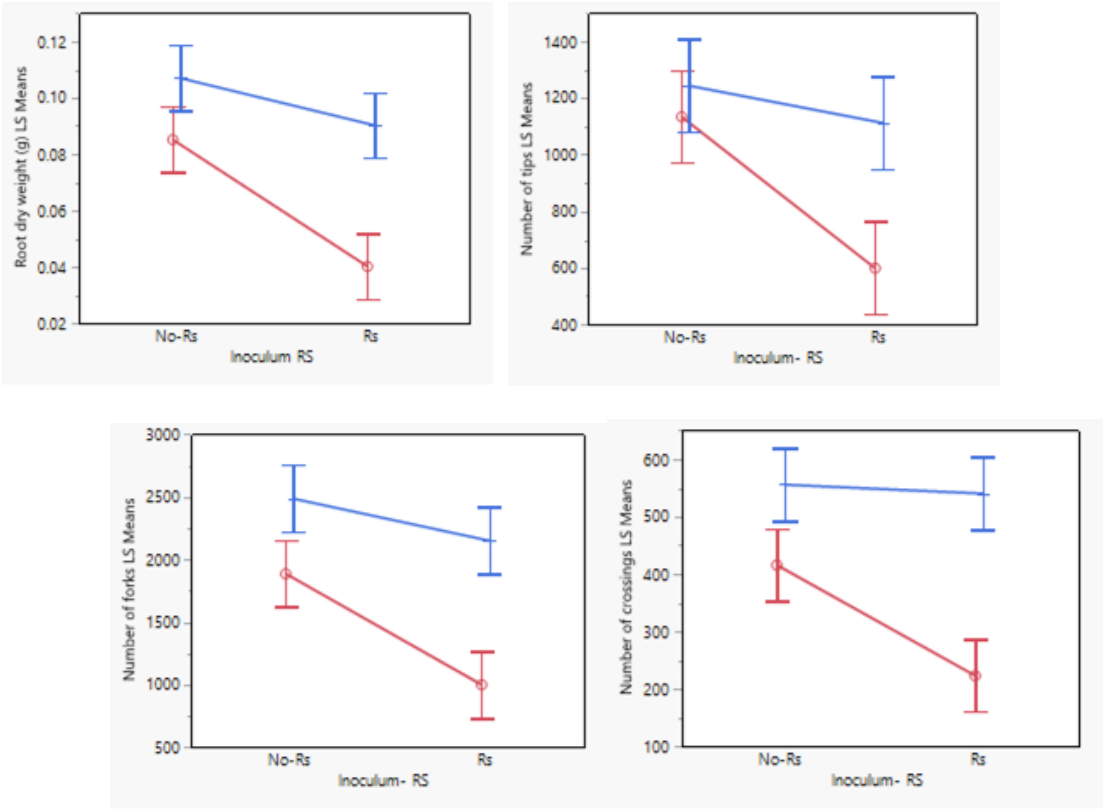

Figure 7. Synergistic effects between soybean seed quality and *Rhizoctonia solani* (Rs) infestation. Root dry weight (A), number of tips (B), forks (C) and crossings (D) of soybean seedlings were affected for the interaction between low vigor seeds and soilborne fungus *R. solani* at 20 ℃ ($p < 0.05$, Least Squares Means Test)

Seed Quality

In this work, the results strongly suggest that soybean seeds with physiological quality problems as well as the occurrence of seedborne pathogens, specifically Rhizoctonia solani, affected root morphological characteristics at 20°C. Synergistic effects between seed aging and Rhizoctonia solani infestation impacted root biomass (root dry weight) and the numbers of root tips, forks, and crossings.

In addition, multiple parameters such as plant length and fresh weight, fresh root weight, root length, volume, and surface area were significantly affected by both seed quality and fungal inoculum.

This research will contribute to determining the impact of environmental conditions and stress factors on the epidemiology of soilborne pathogens that affect seedlings and developing steps toward effective management.

Table 2. Analysis of variance indicating the effects of soybean seed quality and *R. solani* inoculum on plant length (PL), plant weight (PW), root length (RL), root weight (RW) total root length, root surface and root volume .

Effect	df	PL	PW	RL	RW
Seed quality (S)	3	<0.0001	<0.0001	<0.0001	<0.0001
Inoculum (I)	3	<0.0001	<0.0001	<0.0001	<0.0001
(SxI)	3	0.6618	0.5156	0.8712	0.6821
			ROOT		
Effect	df	Length	Surface	Volume	
Seed quality (S)	3	<0.0001	<0.0001	<0.0001	
Inoculum (I)	3	<0.0001	<0.0001	<0.0001	
(SxI)	3	0.2111	0.5047	0.7975	

Discussion

Our results fully support that there is a direct proportional relationship between seed development temperature and seed viability.

Aging-tested seeds showed better development at 25 C. However, even at the same temperature, the disease resistance of seeds without aging test was much higher.

The importance of abiotic conditions when the seed is exposed to a biotic stress factor was not observed in this study.

With this study, one of the reasons for the storage problem, which is one of the biggest problems in soybeans, has attracted attention. A seed that is stored under wrong conditions and has a prolonged storage period, even if the growth conditions are very good, when faced with a soil-borne disease, its resistance decreases.

For this reason, seeds should be stored under appropriate conditions, certified seeds should be used and disease-free planting areas should be preferred.

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