American Journal of Plant Sciences, 2017, 8, 53-68
http://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Effect of Flood Stress on Soybean Seed Germination in the Field

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How to cite this paper: Wu, C.J., Chen, P.Y., Hummer, W., Zeng, A. and Klepadlo, M. (2017) Effect of Flood Stress on Soybean Seed Germination in the Field. *American Journal of Plant Sciences*, **8**, 53-68.
http://dx.doi.org/10.4236/ajps.2017.81005

Received: December 3, 2016
Accepted: January 6, 2017
Published: January 9, 2017

Abstract

Flooding is an abiotic stress that impacts soybean [*Glycine max* (L.) Merr.] growth and reduces seed germination. Effect of flooding on soybean plant grown at different growth stage has been previously conducted and reported. However, soybean seed germination responses to flood stress are largely unknown. The objective of this study was to elucidate flooding influence on soybean seed germination after planting in the field. The research showed that seed germination rate (SGR) of each genotype, without flood stress, was significantly different and ranged between 64.7% to 84.0% and 69.0% to 90.7% while using untreated and fungicide-treated seed ($P < 0.0001$), respectively. Results indicated that fungicide treatment improved soybean seed survival and germination in the field. The average of SGR of high-yielding soybean group was significantly higher than those of non-high-yielding soybean ($P < 0.0001$). The results indicated that high-yielding trait of each genotype was correlated with seed germination and survival. Under flood stress in the field, SGR means of untreated and fungicide-treated seed significantly decreased over eight flooding treatment times ($P < 0.0001$). Flooding effect on germination between untreated and fungicide-treated seed was not significantly different ($P = 0.1559$). Furthermore, comparing the high-yielding and flood-tolerant soybean groups showed no difference in their SGR means over eight flooding treatment times ($P = 0.7687$ and $P = 0.8490$), indicating that soybean seed germination did not depend on genotype, yield, and flood tolerance trait, and seed treated by fungicide did not increase its germination in the field under the flood stress. Hence, it is necessary to develop new soybean seed pelleting to improve seed germination in the field under flooding conditions.

Keywords

Soybean, Flood Stress, Seed Germination Rate, Fungicide Apron Maxx RTA, Yield Trait Group, Flood Trait Group

1. Introduction

Flooding is one of the most hazardous natural occurrences caused by heavy rains, excessive irrigation, and low infiltration rate of soils, and its prolonged appearance severely reduces productivity of crops in major growing regions in the world. Flooding imposes a severe selection pressure on plants since excess water in the living surroundings can deprive plants of oxygen, carbon dioxide, and light [1]. Submerged plant shoots have a severely reduced photosynthesis level due to deficiency of external carbon dioxide causing progressive leaf chlorosis [2]. Root and shoot growth was also affected, and eventually the accumulation of dry matter and seed yield were reduced by flood [3]-[10]. Soybean is an important crop, which is widely used to provide protein, oil, carbohydrates, minerals, and other nutrients for humans and animals [11]. Most soybean cultivars are sensitive to flood stress causing chlorosis, necrosis, stunting, defoliation, reduction of nitrogen fixation, and plant death [12] [13] [14] [15] [16]. All of these symptoms occur at various vegetative (V) and reproductive (R) stages of the plant growth causing various level of yield deterioration [14] [16] [17] [18] [19]. Flooding as an abiotic stress causes approximately 16% reduction in soybean productivity worldwide and loss of billions of dollars for farmers [3] [20] [21]. Flooding regularly affected soybean growth and grain yield around the world including the United States [17] [22]. In the Mississippi Delta region, flood stress can reduce overall soybean yield up to 25% [23]. Previous studies focused on understanding flooding influence on different soybean growth stages and yield cutback. Rhine *et al.* [24] concluded that soybean plants exposed to flood at R5 stage showed yield reduction of 20% - 39% in comparison to non-flooded control. Oosterhuis *et al.* [17] showed that flooding reduced soybean yields by 17% - 43% at the vegetative growth stage, and 50% - 56% at the reproductive stage. Scott *et al.* [18] demonstrated that only 2 days of flooding caused 18% of yield loss at late vegetative stage while it may exceed to 26% if flooding occurs at early reproductive stage of soybean and daily yield reductions are up to 1.6% at V4 and 3.6% at R2 stage. Similarly, Sullivan *et al.* [25] reported a 20% yield loss when soybean plots were flooded for three days at V2 and V3 growth stages. Shannon *et al.* [26] revealed 40% yield reduction in a soybean flood-tolerant group versus 80% reduction in a flood-susceptible group. These previous studies also demonstrated that soybean yield losses were the result of plant death due to occurrence of diseases, physiological stress mostly caused by hypoxia, reduced root and shoot growth, nodulation and nitrogen fixation, photosynthesis, biomass accumulation, and stomatal conductance [16] [17] [23] [27]. However, in contrary to soybean flood tolerance research with focus on plant injury and yield loss, the effect of flood stress on seed germination has not been determined. Hence, it is useful to not only investigate soybean plant growth, but also understand seed germination response to flood stress.

Seed germination is a critical developmental phase in plant life cycle and reproductive success [28]. In general, seed germination capacity is determined by genetic factors and environmental cues such as light, water, temperature, drought, and oxygen [29] [30] [31] [32] [33]. Under flooding, soybean seeds have poor survival and germination in the field due to quick loss of viability in hypoxic environment because oxygen supply is required for germination activation [34] [35] [36]. On the other hand, presence of soil-borne diseases caused by *Phomopsis*, *Pythium*, *Phytophthora*, *Rhizoctonia*, and *Fusarium* significantly impacted on soybean seed germination and seedling emergence [37] [38] [39] [40]. Using seeds covered by an appropriate fungicide increases seed germination of about 10% what results in a large plant emergence in the field [41]. Apron Maxx RTA (Syngenta Crop Protection Inc.) is a broad-spectrum fungicide widely used in the United States for seed treatment, and can control or suppress pathogens *Phomopsis*, *Pythium*, *Phytophthora*, *Rhizoctonia*, and *Fusarium* [42]. However, it is still unknown if this fungicide can maintain soybean seed survival and improve seed germination in the flood-affected field. Therefore, the objective of this study was to investigate the effect of flood stress on the soybean seed germination in the field and identify if broad-spectrum fungicide Apron Maxx RTA can protect seed survival and improve seed germination rate under flooding condition. Knowledge of seed germination interaction with flooding can help plant breeders develop lines and/or cultivars with increased seed germination and seedling growth at high water stress.

2. Materials and Methods

2.1. Plant Materials

A total of 20 soybean genotypes including flood-tolerant (T), flood-moderately-tolerant (MT), and flood-sensitive (S) traits were used as experimental materials, of which, were selected from soybean breeding program at University of Arkansas (**Table 1**). Based on our previous yield trait data (not shown), 20 genotypes also were grouped into high-yielding (HY) and non-high-yielding traits (NHY) (**Table 1**).

2.2. Seed Treatment

Seeds of each genotype were separated two parts untreated and treated by fungicide Apron Maxx RTA with active ingredients Fludioxonil (0.73%) and Metalaxyl-M and S-isomer (1.10%) (Syngenta Crop Protection Inc.). Every 4.540 kg seeds were added 14.8 ml Apron Maxx RTA and mixed even in bucket. The untreated and treated seeds were packed 100 seeds per envelope for planting.

2.3. Seed Planting, Flooding Treatment, and Germination Data Collection

A total of sixteen blocks were planted in the field experiment in 2016, at the Rice Research Experiment Station at Stuttgart, Arkansas (34˚30'N, 91˚33'W). Levees were made to isolate each field block. In each block, all 20 genotypes were planted in a randomized complete block design (RCBD) with three replications. Soybean plants were grown on a crowley silt loam (fine, montmorillonitic, thermic TypicAlbaqualfs). A total of 100 seeds for each genotype were planted in a

Table 1. Twenty soybean genotypes with name, pedigree, flood trait, and yield trait. Foliar damage score (FDS) and plant survival rate (PSR%) of each genotype at R1 stage under flood stress in untreated and fungicide-treated seed tests.

Name	Pedigree	Flood Trait*	Yield Trait#	FDS (US)†	FDS (TS)‡	FDS Mean	PSR% (US)†	PSR% (TS)‡	PSR% Mean
Walters	Forrest × Narow	T	NHY	2.3	2.8	2.6	80.1	78.9	79.5
R10-4892	5002T × R01-3474F	T	NHY	2.2	2.6	2.4	81.2	79.5	80.4
R07-6669	Lonoke × R00-33	T	NHY	2.8	2.6	2.7	78.3	80.2	79.3
UA 5615C	5002T × R04-357	T	HY	1.9	2.2	2.1	86.9	84.3	85.6
R11-6870	5002T × R01-3474F	T	HY	2.6	2.4	2.5	79.6	82.2	80.9
R04-342	R97-1650 × 98601	T	NHY	3.1	2.8	3.0	72.1	75.6	73.9
R13-12552	5002T × 91210-350	T	NHY	3.2	3.1	3.2	71.5	72.4	72.0
Osage	Hartz 5545 × KS4895	MT	HY	4.6	5.0	4.8	58.6	50.5	54.6
UA 5612	R97-1650 × 98601	MT	HY	4.3	4.7	4.5	59.8	54.6	57.2
UA 5213C	R98-1523 × 98601	MT	HY	5.3	5.6	5.5	49.1	47.1	48.1
UA 5115C	BA 743303 × R00-684	MT	HY	4.8	4.5	4.7	52.6	56.9	54.8
UA 5414RR	R96-3427 × 98601	MT	HY	5.6	5.4	5.5	44.6	44.6	44.6
UA 5715GT	Lonoke × Hutcheson-RR	MT	HY	5.4	5.7	5.6	46.8	42.3	44.6
RM-22590	N/A	S	NHY	7.7	7.9	7.8	19.8	18.9	19.4
UA 5014C	Ozark × Anand	S	HY	8.4	8.5	8.5	8.7	8.4	8.6
R01-2731F	Caviness × PI 592947	S	NHY	7.6	8.0	7.8	18.7	15.6	17.2
R99-1613F	NKRA 452 × PI 290126B	S	NHY	7.9	7.7	7.8	19.8	19.5	19.7
R09-4095	S01-9265 × R00-1940	S	NHY	8.3	8.1	8.2	10.3	7.9	9.1
R06-4433	Lonoke × P9594	S	NHY	8.2	8.0	8.1	11.2	11.1	11.2
R10-2379	R01-52F × R02-6232F	S	NHY	8.1	7.9	8.0	12.5	12.8	12.7

*Flood trait, T = flood-tolerant, MT = flood-moderately-tolerant, and S = flood-sensitive; #Yield trait, HY = high-yielding, NHY = non-high-yielding; †FDS, foliar damage score; PSR, plant survival rate; US, untreated seed; ‡FDS, foliar damage score; PSR, plant survival rate; TS, fungicide-treated seed.

3-m row with a 0.75-m row spacing. Three days after planting, flood stress was imposed to the field with 5 - 7 cm of water above the soil surface. Eight different durations which were 6, 12, 24, 36, 48, 72, 96, and 120 hours of flooding treatment, were applied in each type. After flooding treatment, water was drained for seed germination. Meanwhile, two control tests without flood stress including untreated and treated seed were performed in the field. Seed germination number of each genotype were recorded four weeks after removing the flooding water. The seed germination number of two control test without flood stress were also collected in same time. The seed germination rate (SGR) was calculated by dividing the number of emerged seedlings obtained at each counting and per 100 seeds.

2.4. Plants Flooding Treatment and Response Data Collection

Once two control tests soybean plants reached R1 stage, flood stress was imposed (12 - 14 cm of water above the soil surface). After 8-day flooding treatment, water was drained. Foliar damage score (FDS) and plant survival rate

(PSR) were recorded immediately after the termination of each flood treatment. FDS was based on a 1 - 9 scale, where 1 and 9 indicated less than 10 and over 85% of the plants showing foliar damage or death, respectively (1 = 0% - 10%; 2 = 11% - 20%; 3 = 21% - 30%; 4 = 31% - 40%; 5 = 41% - 50%; 6 = 51% - 60%; 7 = 61% - 70%; 8 = 71% - 85%; 9 = 86% - 100%) (**Table 1**). Genotypes were also grouped into three categories; flood tolerant when FSD < 4.0, moderately flood tolerant when FSD = 4.0 - 5.9, and flood sensitive when FSD = 6.0 - 9.0. To determine the plant survival rate (PSR), plants were counted in each row before and after flooding.

2.5. Data Analysis

Analysis of variance (ANOVA) was used for means of seed germination rating between untreated and treated seed test, and plant survival rate was performed using JMP Pro 12.1.0 (SAS Institute Inc., 2012). Significant differences among different treatments were calculated using the LSMeans Difference Student's test with a confidence level of $\alpha < 0.05$.

3. Results

3.1. Germination of Untreated and Fungicide-Treated Seed without Flood Stress

In the untreated seed without flood stress test, the SGR of each genotype was variable and ranged from 64.7% to 84.0% (**Table 2**). LSMeans Difference Student's test showed the differences were significant (P < 0.0001). For the yield trait group comparing analysis, the SGR mean (79.3%) of high-yielding genotypes was significantly higher than that (67.4%) of non-high-yielding genotypes (P < 0.0001) (**Figure 1**). Comparing the flood trait group, the SGR mean (74.3%) of flood-tolerant genotypes was significantly different from the SGR mean (77.9%) of flood-moderately-tolerant genotypes and the SGR mean (68.4%) of flood-sensitive (P = 0.0071) (**Figure 1**).

In the fungicide-treated seed without flood stress test, seed germination rates (SGR) of 20 genotypes also largely varied in the field between 69.0% to 90.7% (**Table 2**). LSMeans Difference Student's test showed the differences were significant (P < 0.0001) (**Table 2**). For the yield trait group comparing analysis, the SGR mean (85.3%) of high-yielding genotypes was significantly higher than that (72.8%) of non-high-yielding genotypes (P < 0.0001) (**Figure 1**). Comparing the flood trait group, the SGR mean (78.0%) of flood-tolerant genotypes was significantly different from the SGR mean (84.0%) of flood-moderately-tolerant genotypes and the SGR mean (74.5%) of flood-sensitive genotypes (P < 0.0001) (**Figure 1**).

Comparing SGR means between untreated and fungicide-treated seed without flood stress tests, the SGR mean off ungicide-treated seed test was 79.2% and dramatically higher than that 73.8% of untreated seed test (**Figure 2**). LSMeans Difference Student's test showed that the difference was significant. Likewise, treatment with fungicide Apron Maxx RTA also largely increased the SGR of

Table 2. Seed germination rate (SGR) of twenty soybean genotypes in untreated and fungicide-treated seed without or with flood stress tests.

Name	SGR% (US/N)*	SGR% (TS/N)#	SGR% (US/F)†	SGR% (TS/F)‡
UA 5615C	84.0 a	90.7 a	25.0 a	25.1 a
UA 5612	83.3 ab	87.3 abc	24.3 a	24.7 a
Osage	81.7 abc	86.7 ab	23.7 a	25.4 a
UA 5414RR	78.7 abcd	84.0 bcd	25.1 a	24.0 a
UA 5715GT	78.7 abcd	84.0 bcd	24.5 a	24.6 a
UA 5014C	78.7 abcd	82.7 cd	25.8 a	23.3 a
R11-6870	78.3 abcd	86.7 abc	24.2 a	24.9 a
UA 5213C	78.3 abcd	88.0 abc	25.2 a	23.9 a
UA 5115C	76.3 bcde	83.3 bcd	25.4 a	23.7 a
R07-6669	75.0 cdef	76.3 ef	24.3 a	24.8 a
Walters	73.0 defg	74.3 efg	23.6 a	25.5 a
R10-4892	71.0 efgh	74.3 efg	24.7 a	24.5 a
R04-342	70.3 efgh	74.7 efg	23.2 a	25.9 a
R10-2379	68.7 fgh	72.3 fg	24.8 a	24.4 a
R13-12552	68.3 fgh	69.0 g	23.8 a	25.8 a
RM-22590	68.3 fgh	72.3 fg	25.3 a	23.8 a
R09-4095	67.3 gh	70.3 g	24.9 a	24.3 a
R01-2731F	66.0 gh	72.7 fg	25.0 a	24.1 a
R06-4433	65.3 h	78.7 de	24.2 a	25.0 a
R99-1613F	64.7 h	72.7 fg	25.1 a	24.0 a

*SGR, seed germination rate; US/N, untreated seed without flood stress; #SGR, seed germination rate; TS/N, fungicide-treated seed without flood stress; †SGR, seed germination rate; US/F, untreated seed with flood stress; ‡SGR, seed germination rate; TS/F, fungicide-treated seed with flood stress.

each genotype in the field (Table 2 and Figure 3). In addition, our results showed that high-yielding genotypes had higher SGR in both untreated and fungicide-treated seed control tests without flood stress (Figure 1 and Figure 3).

3.2. Effect of Flood Stress on Untreated Seed Germination

The effect of flood stress on untreated seed germination in the field was showed in Figure 4. The SGR mean without flood stress (flooding treatment time = 0 hour) was 73.8%. At eight different flooding treatment times (6, 12, 24, 36, 48, 72, 96, and 120 hours), the SGR mean of 20 genotypes in each flooding treatment time was 64.9%, 53.9%, 35.6%, 20.7%, 9.3%, 2.4%, 0.5%, and 0%, respectively (Figure 4(a)). LSMeans Difference Student's test showed that these SGR means reductions were significant between each other with flooding time extension ($P < 0.0001$) (Figure 4(a)). ANOVA analysis further confirmed that these SGR means were variable at different flooding treatment time ($P < 0.0001$) (Table 3). In order to analyze each genotype seed germination response to flood

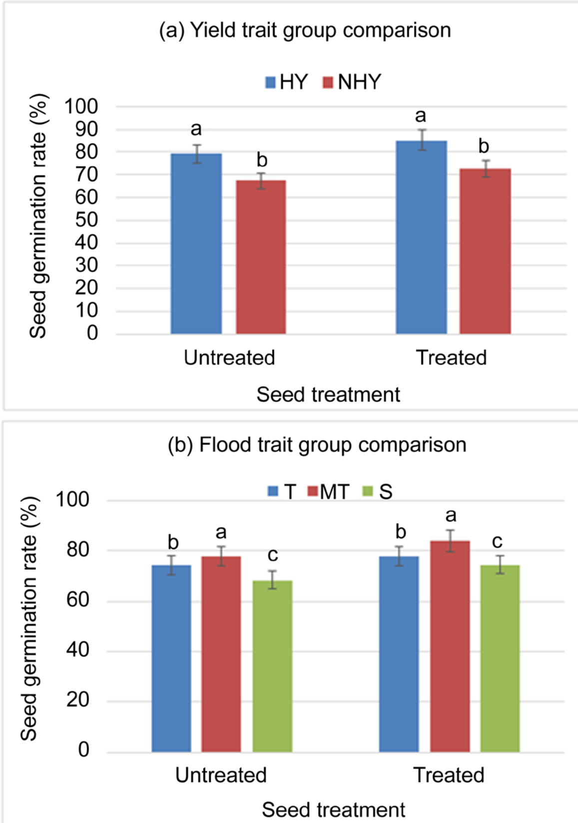

Figure 1. Seed germination rate (SGR) means of untreated and fungicide-treated seed tests without flood stress: (a) Yield trait groups with high-yielding (HY) and non-high-yielding (NHY) traits; (b) Flood trait groups with flood-tolerant (T), flood-moderately-tolerant (MT), and flood-sensitive (S) traits.

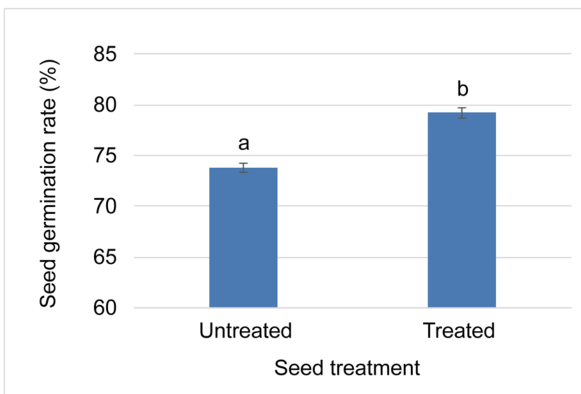

Figure 2. Comparison of seed germination rate (SGR) means between untreated and fungicide-treated seed test without flood stress.

stress, the SGR mean of each genotype over eight flooding treatment times was showed in Figure 4(b). The SGR of each genotype was different and ranged between 19.7% to 26.5% after flooding treatment (Figure 4(b)). LSMeans Difference Student's test showed that these SGR variations among genotypes were not

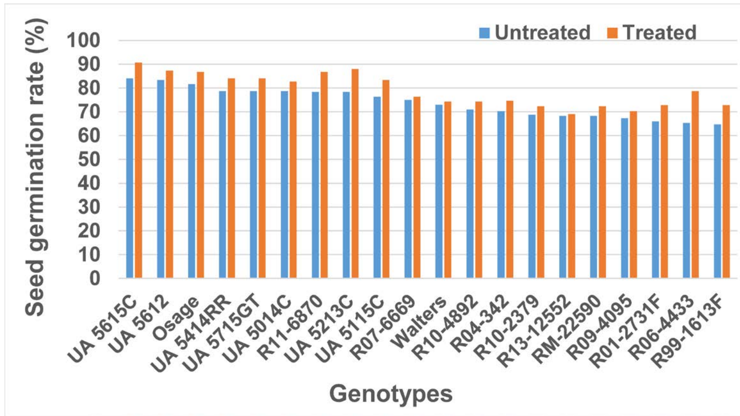

Figure 3. Summary of seed germination rate (SGR) of each genotype between untreated and fungicide-treated seed tests without flood stress.

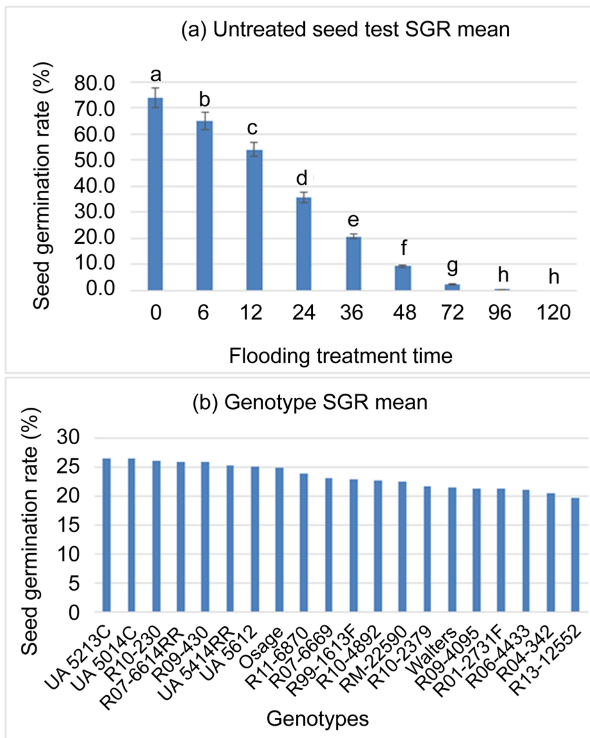

Figure 4. Comparing untreated seed germination rate (SGR) means at different time flood stress: (a) SGR means of untreated seed tests at eight flooding treatment times and control test without flood stress (0 hour flooding treatment time); (b) SGR means of 20 genotypes in untreated seed test over eight flooding treatment times.

significant (P = 0.9999) (Table 2). In addition, the flood stress effect on untreated seed tests revealed that the SGR means of high-yielding and non-high-yielding groups were 25.7% and 21.9% (Figure 5(a)) and the SGR means of flood-tolerant, flood-moderately-tolerant, and flood-sensitive groups were 22.5%, 25.6%, and 22.4%, respectively (Figure 5(b)). LSMeans Difference Student's test showed that these group SGR means were not significant different after flooding (P = 0.2237 and P = 0.9837) (Figure 5).

Table 3. ANOVA analysis for seed germination rates (SGR) of untreated and fungicide-treated seed tests under different flood stress.

Source	DF	Sum of squares	F Ratio	Prob > F
Model†	8	406742.7	2478.4	
Flooding time	8	406742.7	2478.4	<0.0001
Error	531	10893.2		
Total	539	417636.0		<0.0001
Model‡	8	474223.6	3242.1	
Flooding time	8	474223.6	3242.1	<0.0001
Error	531	9708.7		
Total	539	483932.2		<0.0001

†Model, ANOVA analysis for untreated seed germination rates. ‡Model, ANOVA analysis for fungicide-treated seed germination rates.

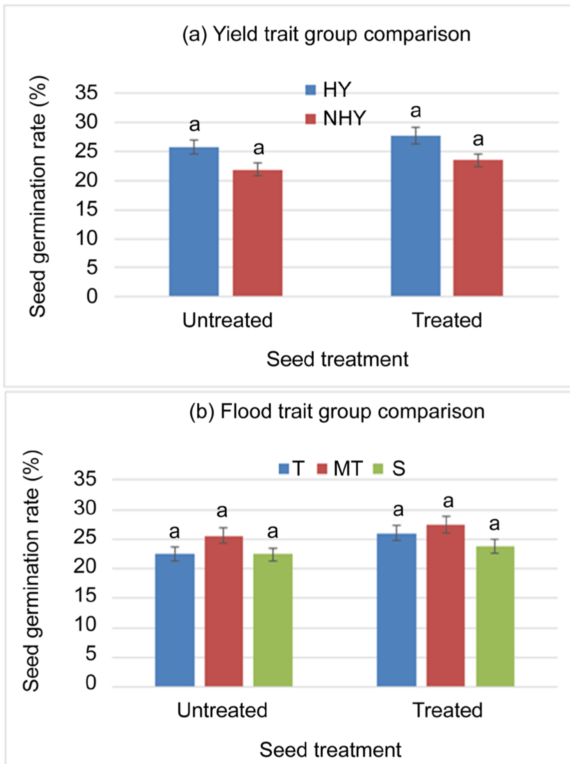

Figure 5. Comparing seed germination rate (SGR) means of different groups over eight flooding treatment times: (a) SGR means of yield trait groups between untreated and fungicide-treated seed tests (HY = high-yielding and NHY = non-high-yielding); (b) SGR means of flood trait groups between untreated and fungicide-treated seed tests (T = flood-tolerant, MT = flood-moderately-tolerant, and S = flood-sensitive).

3.3. Effect of Flood Stress on Fungicide-Treated Seed Germination

The effect of flood stress on fungicide-treated seed germination in the field was showed in Figure 6. The SGR mean without flood stress (flooding treatment time = 0 hour) was 79.2%. At eight different flooding treatment times (6, 12, 24, 36, 48, 72, 96, and 120 hours), the SGR mean of 20 genotypes in each flooding

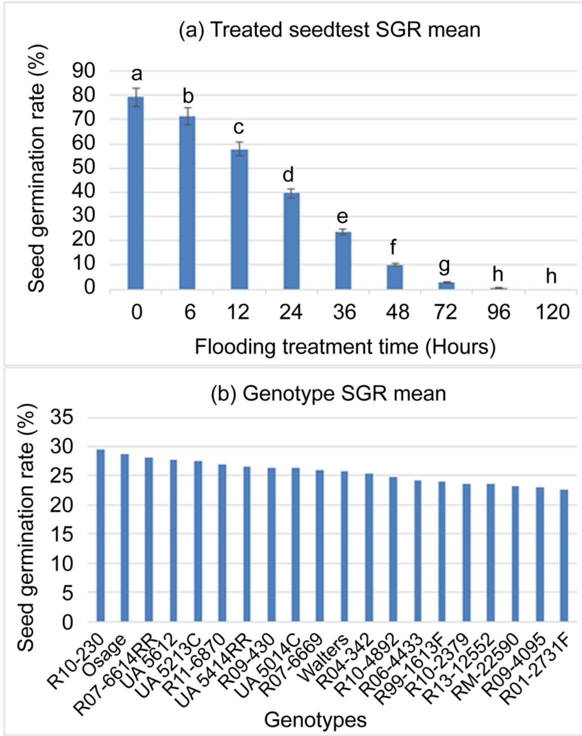

Figure 6. Comparing fungicide-treated seed germination rate (SGR) means at different time flood stress: (a) SGR means of fungicide-treated seed tests at eight flooding treatment times and control test without flood stress (0 hour flooding treatment time); (b) SGR means of 20 genotypes in fungicide-treated seed test over eight flooding treatment times.

treatment time was 71.2%, 57.8%, 39.6%, 23.7%, 10.1%, 2.8%, 0.5%, and 0%, respectively (Figure 6(a)). LSMeans Difference Student's test showed that these SGR means were significantly decreased with flooding time extension (Figure 6(a)). ANOVA analysis further confirmed that these SGR means of fungicide-treated seed test were variations at different flooding treatment time ($P < 0.0001$) (Table 3). The results analysis of each genotype SGR mean over eight flooding treatment times showed that different SGR means between genotypes ranged from 22.7% to 29.6% (Figure 6(b)). LSMeans Difference Student's test showed that these SGR means' variations among genotypes were not significant ($P = 0.9999$) (Table 2). In addition, the flood stress effect on fungicide-treated seed tests revealed that the SGR means of high-yielding and non-high-yielding groups were 27.7% and 23.5% (Figure 5(a)) and the SGR means of flood-tolerant, flood-moderately-tolerant, and flood-sensitive groups were 26.0%, 27.5%, and 23.9%, respectively (Figure 5(b)). LSMeans Difference Student's test showed that these group SGR means were not significant difference after flooding ($P = 0.3928$ and $P = 0.8406$) (Figure 5).

3.4. Germination Rate in Untreated and Fungicide-Treated Seed under Flood Stress

After flooding treatment, the SGR means of untreated and fungicide-treated seed tests over eight flooding treatment times were 23.4% and 25.7%, respectively. LSMeans Difference Student's test analysis showed that two SGR means were not significantly different (P = 0.1559) (**Figure 7(a)**). The SGR mean of each genotype over untreated and fungicide-treated seed tests were shown in **Table 2**. LSMeans Difference Student's test analysis showed that there was insignificant difference of SGR means between untreated and fungicide-treated seed (P = 0.9985) (**Table 2**). Groups comparing analysis showed that the SGR means of untreated and fungicide-treated seed tests were 24.8% and 24.4% in high-yielding group and 24.6% and 24.6% in non-high-yielding group, respectively (**Figure 7(b)**). The SGR means of untreated and fungicide-treated seed tests were 24.1% and 25.2% in flood-tolerant group, 24.7% and 24.4% in moderately

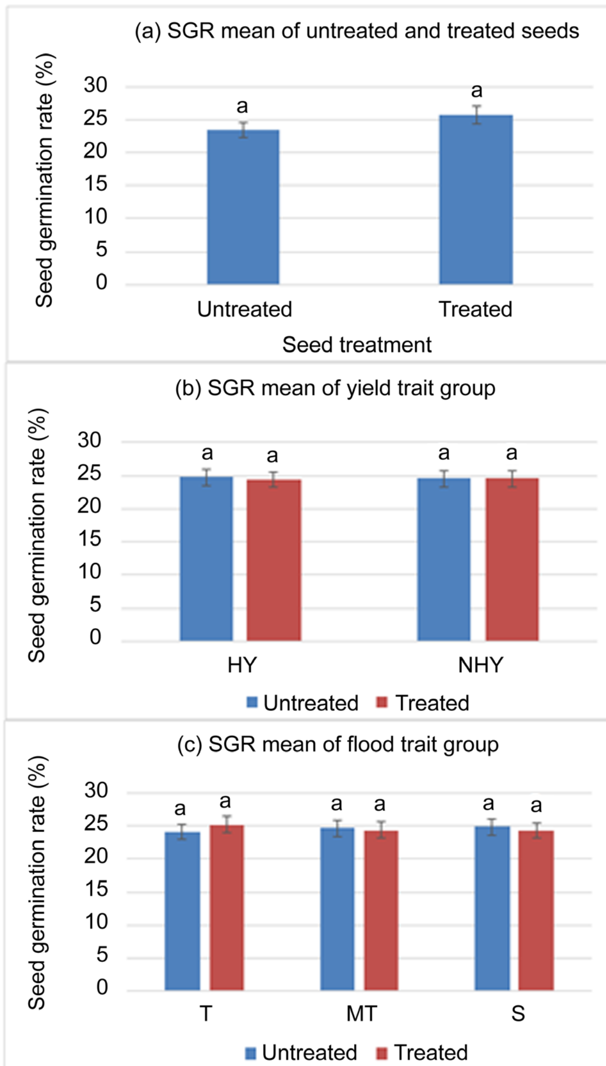

Figure 7. Comparison of seed germination rate (SGR) means between untreated and fungicide-treated seed tests over eight flooding treatment times: (a) SGR means between untreated and fungicide-treated seed tests; (b) SGR means of yield trait groups between untreated and fungicide-treated seed tests (HY = high-yielding and NHY = non-high-yielding); (c) SGR means of flood trait groups between untreated and fungicide-treated seed tests (T = flood-tolerant, MT = flood-moderately-tolerant, and S = flood-sensitive).

tolerant group, and 24.9% and 24.3% in sensitive group, respectively (Figure 7(c)). LSMeans Difference Student's test showed that these groups SGR means were not significant (P = 0.7687 and P = 0.8490).

3.5. Evaluation of Plant Response to Flood Stress

In order to further validate each genotype plant response to flood stress, when plants both untreated and fungicide-treated seed without flood stress tests were grown until R1 stage and then flooded in the field, flood response of each genotype was scored and evaluated. Our results showed that flood responses of 20 genotypes were significantly different in both untreated and fungicide-treated tests (Table 1). For the flood-tolerant group, each genotype showed lower plant foliar damage score (FDS < 4.0) and higher plant survival rate (PSR > 70.0%). Likewise, each genotype showed middle plant foliar damage score (4.0 < FDS < 6.0) and plant survival rate (40% < PSR < 60.0%) in flood-moderately-tolerant group and high plant foliar damage score (FDS > 7.0) and low plant survival rate (PSR < 20.0%) in flood-sensitive group (Table 1). For the high-yielding group, two genotypes UA 5615C and R11-6870 showed flood tolerance, whereas six genotypes UA 5612, Osage, UA 5414RR, UA 5715GT, UA 5213C, and UA 5115C were moderately tolerant to flooding, and one genotype UA 5014C showed sensitivity to flood stress. For the non-high-yielding group, five genotypes including Waters, R10-4892, R07-6669, R04-342, and R13-12552 showed flood tolerance, and six genotypes including RM-22590, R01-2731F, R99-1613F, R09-4095, R06-4433, and R10-2379 were sensitive to flood stress (Table 1).

4. Discussion

Based on previous studies, soybean seed germination rate (SGR) could be reduced by seed disease occurrence caused by soil-borne pathogens [37]-[42]. In our untreated and fungicide-treated seed tests without application of flooding, our results showed that fungicide treatment significantly increased soybean seed germination rate (SGR) in the field. The reasonable annotation is that fungicide provides protection to soybean seeds against damping-off and seed rots due to *Pythium*, *Phtophthora*, *Fusarium*, *Rhizoctonia*, seed-borne *Sclerotinia*, and *Phomopsis* and then improves seed survival, germination, emergence, and seedling stands in the field. In addition, our research also showed that SGR mean of high-yielding group was significantly higher than SGR mean of non-high-yielding group in both untreated and fungicide-treated seed tests without flood stress (Figure 1(a)). It indicates that high-yielding trait of each genotype is linkage to seed germination trait and normally high-yielding soybean cultivars have higher seed germination rate. The genes relevant to seed germination are always selected with high-yielding genes in soybean breeding and domestication. Furthermore, the comparison of SGR means between flood trait groups showed that moderately-flood-tolerant group had the highest SGR mean, the SGR mean of flood-tolerant group ranked second, and flood-sensitive group had minimum SGR mean (Figure 1(b)). This result addressed that soybean seed germination trait is not linkage to flood trait. On the other way, six genotypes of moderately-flood-tolerant group in this research all had high-yielding trait (Table 1). It was a reason that moderately-flood-tolerant group had highest SGR mean in our test and further confirmed that genes relevant to seed germination were linkage to high-yielding genes in soybean. Hence, soybean breeders should cross high-yielding varieties to flood-tolerant varieties and then select varieties with high-yielding and flood-tolerant traits adapting to flooding environment in soybean breeding process.

In this research, we evaluated different flood stress effect to soybean untreated seed germination in the field. When flooding treatment time was extended, the SGR dramatically reduced with longer flooding hours. After only 6 hours flooding, SGR significantly decreased 12.1% and continued to drop to 51.8% after 1-day flood stress. After 2-day flooding, SGR declined to less than 10% and almost no germination in the field (Figure 4(a)). Likewise, the different flood stress also largely impacted soybean fungicide-treated seed germination; the SGR also significantly reduced with extending flooding treatment time. After only 6 hours flooding, SGR significantly decreased 10.1% and continued to drop to 50.0% after 1-day flood stress. After 2-day flooding, SGR declined to less than 10% and almost no germination in the field (Figure 6(a)). Our results showed that flood stress significantly affected soybean seed germination in both untreated and fungicide-treated seed tests in the field. The longer flooding and the less soybean seed germination. One day flooding after planting will result to about 50% seed no germination and soybean yield dramatically reduce. Hence, heavy rain and excessive irrigation in planting season have a large affection to soybean germination and production.

Our research also further showed SGR of each genotype largely decreased with flooding prolong. Each genotype SGR means over eight flooding treatment times between untreated and fungicide-treated seed tests were not significantly different (Table 2, Figure 4(b) and Figure 6(b)). Comparing yield trait group, SGR means of high-yielding group were not significantly different from SGR means of non-high-yielding group in both untreated and fungicide-treated seed tests over eight flooding treatment times (Figure 5(a)). For the comparison of flood trait group, SGR means of flood-tolerant, moderately-flood-tolerant, and flood-sensitive were also not significantly different in both untreated and fungicide-treated seed tests over eight flooding treatment times (Figure 5(b)). These results indicated that soybean seed germination was not significantly affected by genotype, yield, and flood trait under the flood stress. In other words, soybean genotype with yield and flood traits cannot significantly increase seed germination in the field under flood stress.

Comparing flood stress effect between untreated and fungicide-treated seed tests over eight flooding treatment times, the SGR means were not significantly different (Figure 7(a)). For the yield trait group, the SGR means of high-yielding group between untreated and fungicide-treated seed tests were not significantly different and SGR means of non-high-yielding group between untreated and fungicide-treated seed tests were also not significantly different (**Figure 7(b)**). For the flood trait group, the SGR means of flood-tolerant, moderately-flood-tolerant, and flood-sensitive groups were not significantly different between untreated and fungicide-treated seed tests (**Figure 7(c)**). These results further indicated that soybean seed treated by fungicide Apron MaxxRTA did not increase SGR under flood stress in the field. The damage effect of flooding is the same to untreated and fungicide-treated soybean seeds in the field. The fungicide Apron MaxxRTA cannot effetely protect soybean seed survival and increase germination under flood stress. Therefore, it is necessary to develop new seed pelleting and improve seed germination on flooding environment.

Acknowledgements

We would like to thank the United Soybean Board and Arkansas Soybean Promotion Board for providing the financial support. We also thank Jonathan McCoy at the Rice Research and Extension Center in Stuttgart, AR for his support and assistance.

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