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Chlorophyll Fluorescence, Oxygen Consumption Rates and Germination of Green Soybean Seeds Produced Under Heat–Drought Stress

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ABSTRACT

Heat–drought stress during the late development of soybean seeds (*Glycine max* [L.] Merr.) adversely affects chlorophyll degradation, resulting in green seeds with low physiological quality. This study aimed to relate chlorophyll fluorescence, oxygen consumption rates, and germination characteristics in green and yellow soybean seeds produced under heat–drought stress conditions. Seeds produced under favorable growth conditions were used as controls. Seed chlorophyll fluorescence was measured as well as individual seed respiratory activity by measuring oxygen levels during germination over 90 h at 25°C. Results indicated that green seeds, with the highest chlorophyll fluorescence, exhibited the lowest initial metabolic rates and germination percentages. Additionally, green seeds took longer to consume 50% of the available oxygen, requiring 45.90 h compared to 25.54 h for yellow seeds and 19.63 h for control seeds. Germination rates and embryonic axis lengths were significantly lower for green seeds (11.1% germination and 0.59 cm length) compared to yellow seeds (65.4% and 1.04 cm) and control seeds (83.3% and 1.44 cm). A negative correlation was found between chlorophyll fluorescence and both metabolic rates and embryonic axis length, indicating that heat–drought stress severely impacts chlorophyll degradation, oxygen consumption, metabolic rates, and germination in green soybean seeds.

1 | Introduction

The retention of chlorophyll (Chl) in mature soybean (*Glycine max* [L.] Merr.) seeds, commonly known as green seed (GS), has been reported in Argentina and Brazil since the 2000s (Sinnecker 2002; Craviotto and Arango 2005; França-Neto et al. 2005; Mandarino 2005). The presence of GS is mainly associated with high temperatures and low rainfall during late maturation (heat–drought stress [HDS]), which negatively affects the oil and physiological qualities of these seeds (Pádua et al. 2007; Gallo 2008; Cencig 2013; Teixeira et al. 2016). The Chl level when the seeds are fully formed (stage R6 according to Fehr and Caviness 1977; 70% moisture content) is approximately 500 mg.kg^{-1}. At physiological maturity (R7; 54%–62% moisture content) the maximum dry weight is reached (TeKrony et al. 1979; Sinnecker et al. 2005), and the Chl breakdown by the action of Chl degrading enzymes occurs in a multistep pathway (Hörtensteiner and Kräutler 2011). HDS conditions during R5.5–R7 cause rapid seed dehydration, thus affecting enzymatic and respiratory activity and resulting in high percentages of GS. The residual Chl levels are related to the severity of the stress and genotype susceptibility (Pádua, França-Neto, et al. 2009; Cencig 2013; Teixeira et al. 2016) and determine the total or partial coloration in these mature GS. Several studies have shown that the presence of GS is negatively correlated with seed quality (Gallo 2008; Cicero et al. 2009; Cencig 2013). Chl fluorescence measurement is a nondestructive technique explored to analyze and classify seeds individually according to the Chl content. Chl fluorescence occurs when part of the light absorbed by a green plant or organ (including seeds) is re-emitted over a longer wavelength, usually between 650 and 750 nm. Chl fluorescence has been used as an indicator of maturation and seed quality in soybeans (*G. max* L.) (Cicero et al. 2009), cabbage (*Brassica oleracea*) (Dell'Aquila et al. 2002; Jalink et al. 1998), tomato (*Solanum lycopersicum*) (Jalink et al. 1999), barley (*Hordeum vulgare*) (Konstantinova et al. 2002), carrot (*Daucus carota*) (Groot et al. 2006), pepper (*Capsicum annuum*) (van der Burg 2008), and rice (*Oryza sativa* L.) (Hay et al. 2015). Seed germination sensu stricto initiates with the resumption of water uptake and culminates in the radicle protrusion through the seed covering layers (Bewley et al. 2013). Respiration is an early metabolic process that begins at imbibition. Therefore, measuring the real-time oxygen consumption of seeds can directly indicate their metabolic status during germination and reveal fundamental relationships between respiration and germination rates (Baker et al. 2004; Bello and Bradford 2016; Huang and Taylor 2019). The Seed Respiration Analyzer (SRA) can measure the oxygen consumption of a single seed with detailed time resolution. The measured curves can be used to evaluate seed quality. Oxygen consumption rate during seed germination is a promising parameter for monitoring seed status and predicting germination and seed vigor. It is essential to be aware of how soybean seed physiological quality can be negatively affected so that management practices and new technologies can be applied to reduce the risk of poor-quality seed lots. In that sense, several studies have described the inverse relationship between Chl retention and germination rates (Jalink et al. 1999; Cicero et al. 2009). However, there is currently no clear evidence on the influence of the stressful growing conditions on the Chl retention and how this affects the physiological and metabolic characteristics of GSs. The objective of the present work was to relate Chl fluorescence, oxygen consumption rates, and germination characteristics of soybean seeds with Chl retention cultivated under HDS.

2 | Materials and Methods

2.1 | Plant Material and Grow Conditions

Soybean seeds (*G. max* [L.] Merr.) of the cultivar SRM 3410 were selected due to their higher susceptibility to GS production during natural HDS in the field (Enrico et al. 2021). High-quality seeds of the cultivar SRM 3410, produced under favorable growth conditions, were sown and plants were grown in the greenhouse (Plant Physiology Laboratory, FCA-UNR, Zavalla, Argentina, at 33°01′ South latitude and 60°53′ West longitude) in 5-dm^3 pots filled with 3:1 (v/v) humus-rich soil and perlite. Pots were overseeded, and at the unifoliate leaf stage, seedlings were thinned to obtain two uniform seedlings per pot. The average temperatures during day and night were 25°C ± 4°C and 15°C ± 4°C, respectively. From the reproductive phase R5.5 to R8, half of the pots were set under HDS in the greenhouse. Plants under HDS were only watered when turgor loss was observed to avoid wilting. The rest of the pots were placed outdoors, exposed to the local environmental conditions and freely watered (field capacity) for the nonstressed treatment (NS), considered as control. During R5.5–R8, the mean and maximum temperatures were 26.6°C ± 4.6°C and 33.8°C ± 5.9°C for the HDS treatment and 24.8°C ± 3.6°C and 30.6°C ± 3.7°C for the NS, respectively (Figure 1). The seed lots were hand-harvested at R8. The percentage of GS was determined using four replicates of 100 seeds from the HDS and NS treatments. For further studies, the seed samples obtained from the HDS treatment were divided based on visual analysis into GS (visible Chl retention) and yellow seeds (YS—nonvisible Chl retention). The NS seed samples were used as control seeds (CS) (Figure 2). Samples were stored at 4°C in a seed store until the study was conducted.

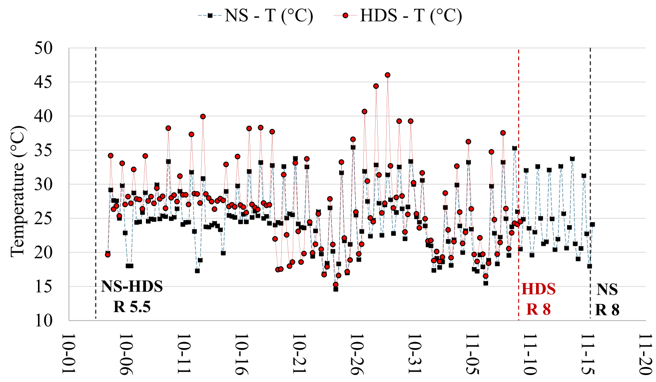

FIGURE 1 | Average mean daily temperature (°C) at the reproductive phase R5.5-R8 of the soybean seed production for the nonstressed (NS, --■--) and heat–drought stress (HDS, —●—) treatments.

2.2 | Tetrazolium Vigor Test

Two replicates of 50 GS, YS, and CS each were used, preconditioned on paper towels moistened with distilled water, then placed in plastic bags to prevent loss of moisture and incubated at 20°C ± 2°C for 18 h in the dark. Seeds were stained in a 0.1% (w/v) solution of 2,3,5-triphenyl-tetrazolium chloride and incubated in a thermostatic bath at 35°C ± 2°C for 3 h in the dark. After staining, the seeds were washed in tap water and classified into Categories A (without defects), B (minor defects), and C (moderate defects) and other staining (severe defects) and nonviable. Mean percentages for viability were calculated from the sum of Categories A–C and other staining, and for vigor, from the sum of Categories A–C, according to the methodology described in the Handbook Topographic Tetrazolium Test for Soybean (Craviotto et al. 2008) and ISTA Rules (ISTA 2022).

2.3 | Chl *a* and *b* and Total

Fifty GS, YS, and CS each were ground for the determination of Chl content ($mg.L^{-1}$). The extraction of Chl and the quantification of Chl *a* and *b* and total was carried out according to Arnon (1949). Three biological replicates of 1 g of seed tissue of GS, YS, and CS each were homogenized with 10 mL of 80% (v/v) acetone at 4°C in the dark for 48 h. The samples were centrifuged for 5 min at 4000 rpm, and the supernatants were extracted in sterile tubes, and the Chl content was measured on 1-mL aliquots by using a

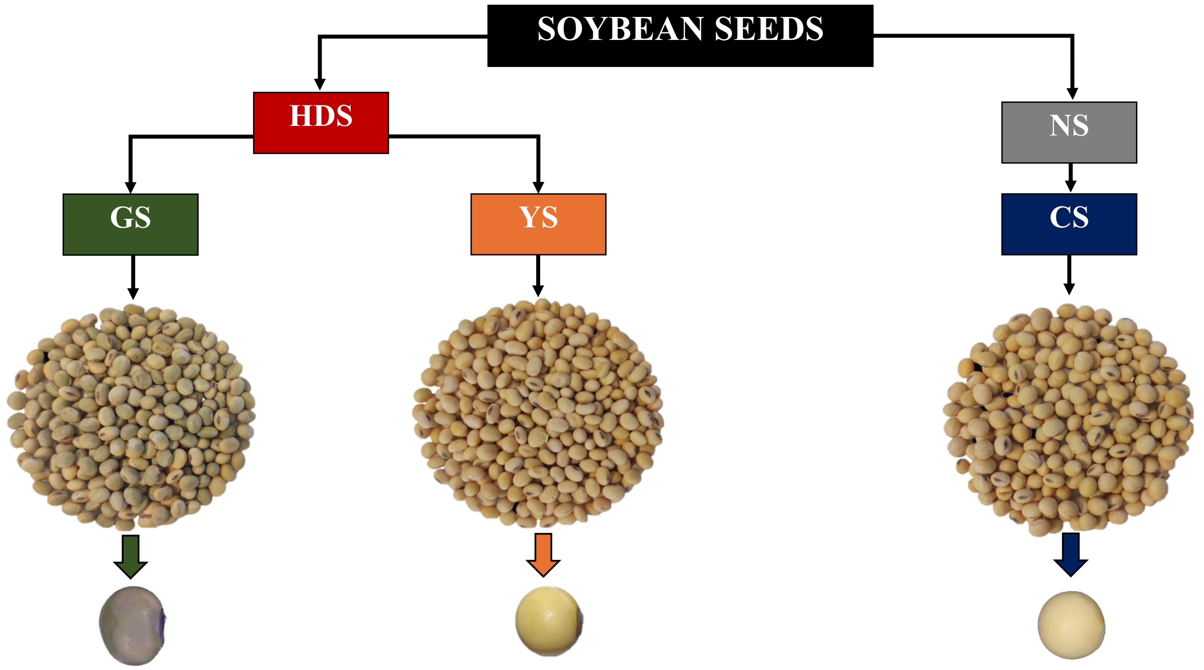

FIGURE 2 | Soybean seeds of the cultivar SRM 3410 produced under heat–drought stress (HDS) were manually separated into green (GS—visible chlorophyll retention) and yellow seeds (YS—nonvisible chlorophyll retention), and nonstressed (NS) seeds were used as control (CS).

spectrophotometer BioRad SmartSpec 3000 at the wavelengths of 663 and 645 nm, corresponding to the absorption peaks of Chl *a* and *b*, respectively. The Chl *a* and *b* and total content were calculated according to the following equations (Arnon 1949):

$$\text{Chl } a = [(12.7 \times \text{Abs } 663) - (2.69 \times \text{Abs } 645)],$$

$$\text{Chl } b = [(22.9 \times \text{Abs } 645) - (4.68 \times \text{Abs } 663)],$$

$$\text{Chl total} = [(20.2 \times \text{Abs } 645) + (8.02 \times \text{Abs } 663)].$$

2.3.1 | Chl Fluorescence

A nondestructive method was used to measure the individual Chl fluorescence in seeds using a modified Waltz Chl Fluorescence sensor type Moni-Head CFMD0123 (Effeltrich, Germany). The sensor was equipped with a seed holder. The Chl fluorescence was measured 10 times at different parts on 30 individual GS, YS, and CS each. A light beam with a wavelength of 670 nm was used to excite the Chl, and the Chl fluorescence ($\lambda = \sim 720$ nm) was collected by a photodiode with filters to intercept the excitation light and allow the emitted Chl light to pass (Hay et al. 2015). The results were expressed as the intensity of the minimum Chl fluorescence signal (f0), the maximum Chl fluorescence signal (fm) after an oversaturation excitation pulse, and the photosynthetic activity (fv/fm = (fm − f0)/fm).

2.3.2 | Single Seed Oxygen Consumption During Germination

The SRA instrument was used as a nondestructive technology to measure oxygen consumption of individual seeds during germination (https://www.fytagoras.com/en/seed-respiration-analyzer/). Thirty GS, YS, and CS each were weighed individually

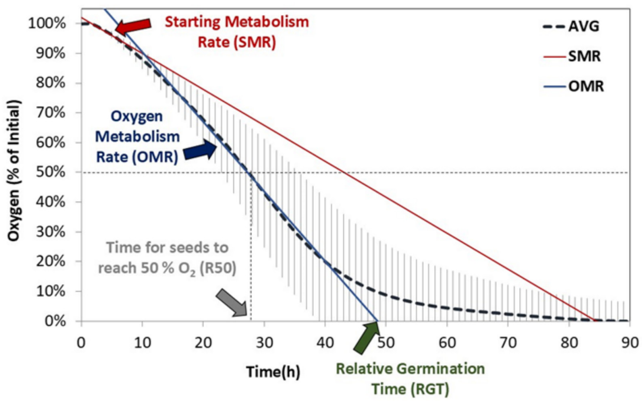

FIGURE 3 | Average (AVG) oxygen depletion curve with bars representing the standard error as processed by the mathematical model Q2 Analysis to calculate the starting metabolism rate (SMR, % O_2.h^{-1}.g^{-1}), oxygen metabolism rate (OMR, % O_2.h^{-1}.g^{-1}), time for seeds to reach 50% O_2 (R50, h), and relative germination time (RGT).

(g) and placed on 1000-μL agar (0.4% w/v) in individual 5-mL screw-capped tubes containing an oxygen-sensitive fluorescent coating. Individual seed oxygen (O_2) consumption was measured at 30-min intervals during 90 h of incubation at 25°C ± 2°C. The initial O_2 concentration (21%) was normalized to 100% based on the first measurement for each individual tube. Oxygen consumption rates were expressed as a percentage of O_2 consumption per hour and per gram of seed (% O_2.h^{-1}.g^{-1}). For the SRA data, starting metabolism rate (SMR, % O_2.h^{-1}.g^{-1}), oxygen metabolism rate (OMR, % O_2.h^{-1}.g^{-1}), time for seeds to reach 50% O_2 (R50, h), and relative germination time (RGT) were calculated from the O_2 depletion curves using the mathematical model Q2 Analysis (Department of Plant Science, University of California, Davis; Figure 3). After the SRA experiment, seeds were extracted to determine the individual germination and the embryonic axis length (AL, cm). Total germination was expressed in % G, and AL (used as an indicator of growth) was measured by using ImageJ software (Schneider et al. 2012).

2.3.3 | Statistical Analysis

ANOVA test was performed on the results of viability %, vigor %, Chl *a* and *b* and total, f0, fm, fv/fm, SMR, OMR, R50, and RGT and AL to estimate differences between treatments (GS, YS, and CS). Tukey test at the significance level of 5% ($p \leq 0.05$) was used. Before ANOVA analysis, percentage data such as viability % and vigor % were arcsine transformed. These calculations were done with the statistical software InfoStat version 2020 (Di Rienzo et al. 2020).

To explore the associations between f0, fm, fv/fm, SMR, OMR, R50, RGT, and AL, Pearson correlation and principal component analysis (PCA) were performed with the aim of reducing the dimensionality of a multivariate data set while preserving variability. A biplot graph was constructed from the first and second principal components (PC1 and PC2) derived from the PCA, using R Studio software (Version 1.4.1106, RStudio Team).

3 | Results and Discussion

3.1 | Chl Retention in Soybean Seeds Produced Under HDS

The combination of high temperature (> 32°C) and drought stress is considered the main cause of forced seed maturation in soybean, inducing severe damage to seed yield and quality (Pádua 2006; Gallo 2008; Cencig 2013; Teixeira et al. 2016). In our experiment, the HDS in the greenhouse during the late-developing (R5.5–R8) of soybean seeds interfered with the Chl degradation, remaining a majority of seeds green at harvest, thus resulting in a high level of GS reaching 82% with visible Chl retention. In the NS, only 0.01% GS was observed. Under HDS conditions, reserves are rapidly translocated to the seeds, preventing the complete degradation of the Chl and promoting GS production of low quality and reduced performance. On the other hand, the stress may cause the premature death of the plants, affecting the speed of seed maturation and Chl degradation (Teixeira 2014; Pádua 2006). The plants under HDS started the leaf senescence and seed maturation earlier than in NS conditions, reaching R8 at 99 and 105 days, respectively.

In addition, the susceptibility of soybean cultivars to GS constitutes an important determining factor (Pádua, De Moreira Carvalho, et al. 2009, Cencig 2013, Teixeira et al. 2016). In previous studies, the percentages of GS of 11 commercial cultivars were studied in the field for both early and optimum sowing dates during two growing seasons (2017/2018 and 2019/2020). The cultivars SRM 3410, SPS 4X4, and DM 4214 STS showed the highest percentages of GS in both campaigns (Enrico et al. 2021). Moreover, the percentage of GS and the Chl retention are highly dependent on the duration and intensity of the stress conditions, as well as the stage of the crop, even in the same susceptible genotype. Taken all these factors into account, the HDS controlled conditions in the greenhouse could have resulted in a more severe stress and thus explaining the higher GS percentage obtained for SRM 3410 (82%) under these conditions as compared to that for the same cultivar in the field, which were 39.3% and 29.3% GS and 36.3% and 31.1% for early and optimum sowing dates in 2017/2018 and 2019/2020, respectively (Enrico et al. 2021).

3.2 | Tetrazolium Test for Viability and Vigor

The initial physiological quality of the seeds was assessed using the tetrazolium test for viability and vigor. The tetrazolium test is based on the respiratory activity of the seminal tissues after imbibition. This allows the identification of damages caused by the production environment, mechanical, or biotic factors, through staining patterns. Costa et al. (2001) and Pádua et al. (2007) established a negative correlation between GS % and viability and vigor as determined by the tetrazolium test. In addition, Gallo (2008) reported that the GS exhibited less than 20% viability and 10% vigor. Pádua et al. (2007) observed a decline in vigor from a maximum of 82% to 4% as GS levels increased from 0% to 100%.

The average viability and vigor of our study were 58% and 22% for GS, 85% and 75% for YS, and 87% and 78% for CS, respectively (Table 1). The GS showed a significant reduction in viability and vigor, as compared to YS and CS, which could be responsible for the lower seed germination performances. Previously, we studied the %G of seeds produced in the field and in the greenhouse under HDS from cultivars SRM 3410, SPS 4X4, and DM 4214 STS, revealing that GS had a significantly lower germination percentage as compared to YS and CS. For field-produced seeds in the 2017/2018 season, germination percentages were YS 77% and GS 30% (SRM 3410), YS 97% and GS 30% (SPS 4X4), and YS 97% and GS 33% (DM 4214

TABLE 1 | Mean percentages of viability, sum of Categories A (without defects), B (minor defects), and C (moderate defects) and other staining (severe defects) and mean percentages of vigor, sum of Categories A–C, determined by the tetrazolium test of green (GS), yellow (YS), and control (CS) seeds.

	Category A	Category B	Category C	Other staining	Nonviable	Viability	Vigor
GS	4	8	10	36	42	58 ± 2.8 a	22 ± 2.8 a
YS	35	31	9	10	15	85 ± 1.4 b	75 ± 4.2 b
CS	21	17	40	9	13	87 ± 1.4 b	78 ± 2.8 b

Note: Means ± standard deviations followed by the same letter are not significantly different ($p \leq 0.05$), as determined by the Tukey test.

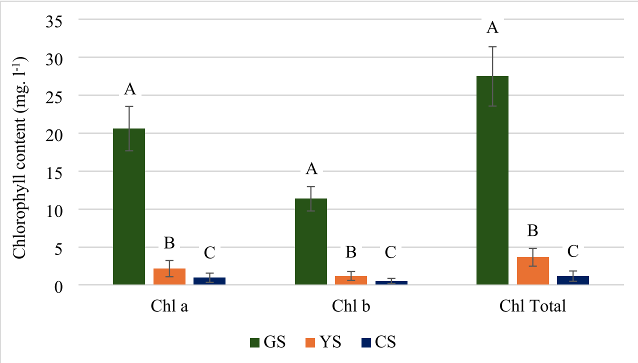

FIGURE 4 | Mean chlorophyll *a* and *b* and total of soybean seeds produced under heat–drought stress (HDS) classified as green (GS) and yellow (YS) and nonstressed, control seeds (CS). Bars represent the mean values ± standard deviations in mg.L^{-1} of three biological replicates. An ANOVA analysis was performed, and means were compared by Tukey test at $p < 0.05$; different uppercase letters indicate significant statistical differences.

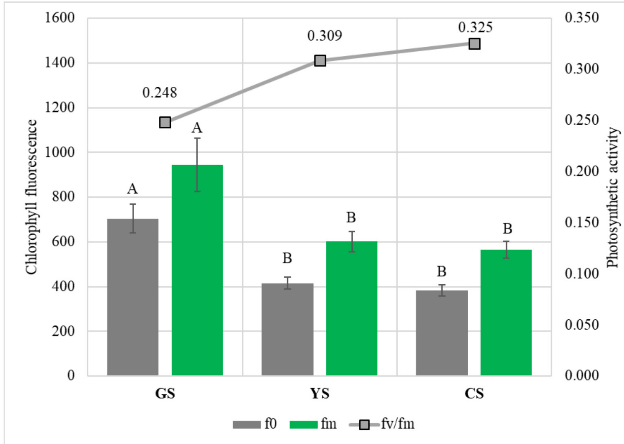

FIGURE 5 | Chlorophyll fluorescence minimum (f0) and maximum (fm) and photosynthetic activity (fv/fm = (fm − f0)/fm) of green (GS), yellow (YS), and control seeds (CS) of the cultivar SRM 3410. Bars represent the mean values ± standard deviations of 10 different measurements on 30 biological replicates of GS, YS, and CS. Letters represent statistically significant differences ($\alpha = 0.01$) by Tukey test.

STS) (Figure S1). In greenhouse conditions, similar differences were observed ($p < 0.05$), with %G for SRM 3410 (CS/YS 87%, GS 27%), SPS 4X4 (CS 100%, YS 97%, GS 33%), and DM 4214 STS (CS/YS 97%, GS 40%). In both scenarios, GS failed to reach a germination rate above 40% (Figure S2). The results indicated that YS produced under HDS conditions were similar to CS, in terms of viability, vigor, and germination, but they were significantly different from GS.

3.3 | Chlorophyll *a* and *b* and Total

Chl degradation reduces possibilities for Chl to produce free electrons, which may cause oxidative injury and subsequent effects on seed viability and longevity. Seed deterioration during storage is often attributed to oxidative stress caused by free radicals (Jalink et al. 1999). The level of Chl retention, considering Chl *a* and *b* and total of the GS, YS, and CS, was measured by spectrophotometry. The content of Chl *a* and *b* and total of the GS (20.61, 11.36, and 27.49 mg.L^{-1}, respectively) was significantly higher than in the YS (2.15, 1.18, and 3.64 mg.L^{-1}, respectively) and CS (0.94, 0.52, and 1.16 mg.L^{-1}, respectively) (Figure 4). The Chl content of both YS and CS was very low, independent of the growing conditions. In addition, lower values of Chl *b* were observed compared to Chl *a*. Previous studies by Sinnecker et al. (2005) and Pádua et al. (2007) in soybean reported Chl *a*:*b* ratios of 1.8:1 and 1.6:1, respectively. In this research, the Chl *a*:*b* ratios for soybean seeds produced not only under normal conditions (CS) but also under HDS (GS and YS) were found to be 1.81:1, aligning closely with Sinnecker et al. (2005), also suggesting that the effect on the degradation process, which is responsible for the green color disappearance, does not affect the Chl *a*:*b* ratios.

3.4 | Chl Fluorescence

It must be emphasized that given the variation in the percentages of GS and the level of Chl, not all seeds have the same metabolic and physiological condition. Consequently, in this study, the seeds were individually characterized in terms of weight, Chl fluorescence, respiration, germination, and embryonic axis growth. The Chl fluorescence of individual seeds was assessed by a nondestructive Chl fluorescence sensor, taking into account the variability that may exist at the level of the seeds. The f0 signal range was 328.1–1064.3 for GS, 357.9–473.0 for YS, and 298.6–466.2 for CS. Meanwhile, the fm signal range was 696.9–1413 for GS, 471.1–722.1 for YS, and 466.5–682.2 for CS. The f0 and fm averages were 703.6 ± 114.4 and 944.2 ± 189.2 for GS, 381.8 ± 52.2 and 601.7 ± 93.0 for YS, and 415.1 ± 65.1 and 566.1 ± 78.3 for CS (Figure 5). Comparing the treatments, the f0 and fm signals were similar between CS and YS but lower with respect to GS.

Researchers have investigated the potential of Chl fluorescence sorting equipment to enhance seed quality. Cicero et al. (2009) analyzed soybean seed samples with GS from 0% to 20% using a Chl fluorescence sensor, classifying them into control, low, and high Chl fluorescence categories. Chl fluorescence levels increased with GS %, ranging from 703.8 to 2556.7 across categories. Germination rates for high Chl fluorescence seeds decreased from 80.0% to 60.5%, while vigor measured by the controlled deterioration test dropped from 52% to 19%. This study concluded that GS exhibit high Chl fluorescence, negatively affecting seed quality. Removing GS through Chl fluorescence sorting can improve soybean seed quality, as demonstrated in similar studies in *B. oleracea* seeds (Jalink et al. 1998).

Photosynthesis in soybean seeds generates ATP and NADPH, which contribute to fatty acid biosynthesis during seed development (Allen et al. 2009; Baud and Lepiniec 2010; Borisjuk et al. 2003; Smolikova and Medvedev 2016). Compared to leaves, photosynthetic activity in seeds is reduced due to shade-adaptive traits, lower levels of Calvin–Benson cycle enzymes, and a diminished Chl *a*:*b* ratio (Borisjuk et al. 2003; Ruuska et al. 2004). During late embryogenesis, seeds undergo dehydration and chloroplast disintegration, though embryonic photosynthesis primarily supports reserve accumulation and oxygen supply (Smolikova and Medvedev 2016).

Photosynthetic activity (fv/fm) is a well-established indicator of the maximum efficiency of Photosystem II and is commonly used in photosynthetically active tissues. However, its interpretation in seeds—particularly dry or maturing ones—requires caution. In soybean seeds, chloroplasts are progressively dismantled during maturation, resulting in the degradation of Chl and the transformation of plastids into nonphotosynthetic forms. Low values of fv/fm were obtained, being 0.248 for GS, 0.309 for YS, and 0.325 for CS (Figure 5). In our study, values remained low even in seeds without spectrophotometrically detectable Chl. In the case of GS, the retained pigmentation does not necessarily reflect active photosynthetic function. Therefore, fv/fm may serve as a complementary indicator of Chl presence or degradation status rather than a direct measure of photosynthetic performance in seeds.

3.5 | Single Seed Oxygen Consumption During Germination

Respiration by mature dry seeds (moisture content < 15%) is markedly reduced in comparison with developing or germinating seeds (Bewley et al. 2013). When a viable seed is imbibed, the completion of germination is contingent upon the generation of ATP and reductants via oxidative mitochondrial respiration. The onset of respiration per se occurs within minutes of the start of imbibition. Initially, there is a sharp increase in O_2 consumption, which can be attributed, in part, to the activation and hydration of mitochondrial enzymes. Respiration increases linearly as more cells within the seed become hydrated. Following the completion of imbibition, a lag in respiration occurs as O_2 uptake is stabilized or increases only slowly (Bewley et al. 2013). Due to damage caused during dehydration, harvesting, storage, and rehydration, several repair mechanisms are activated in imbibition. These processes include the repair of membranes, proteins, and DNA, which are essential for successful germination. The speed and percentage of germination are correlated with respiratory capacity and are among the most sensitive indicators of seed vigor. A reduction in respiration rates was observed in seeds lacking gibberellic acid or exposed to high concentrations of ABA that prevented germination and also in aging seeds. Bello and Bradford (2016) confirmed that seed O_2 consumption rates on an individual seed and population basis are highly correlated with germination timing across a wide range of conditions that affect seed germination rates, including temperature, water potential, hormones, aging, or priming.

For GS, SMR was 0.50% $O_2.h^{-1}.g^{-1}$ within the initial 20 h of incubation, and OMR was 3.27% $O_2.h^{-1}.g^{-1}$ between 45 and 55 h. In contrast, for YS, the SMR was 0.71% $O_2.h^{-1}.g^{-1}$ within the initial 4 h of incubation, and the OMR was 2.38% $O_2.h^{-1}.g^{-1}$ between 10 and 25 h. In CS, the pattern of O_2 consumption was similar to YS, with SMR being 1.39% $O_2.h^{-1}.g^{-1}$ within the first 4 h and OMR being 3.01% $O_2.h^{-1}.g^{-1}$ between 10 and 25 h (Figure 6). GS demonstrated a prolonged time course for the consumption of the initial 50% O_2, reaching R50 at 45.90 h, whereas YS and CS

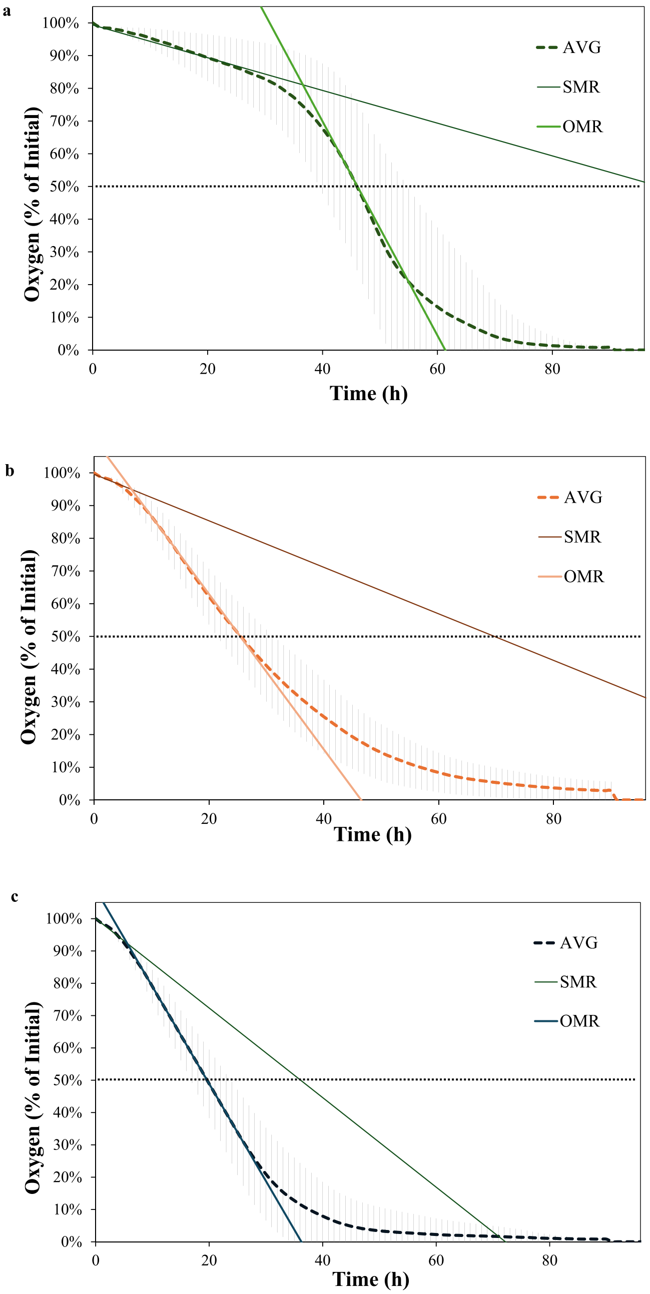

FIGURE 6 | Average (AVG) oxygen consumption of GS (a), YS (b), and CS (c) of the cultivar SRM 3410 (thick dashed lines). Bars represent the standard error for 30 GS, YS, and CS, respectively. The dotted line at 50% O_2 level crossed the O_2 level line at the time to seeds reaching 50% O_2 level (R50, h). The slopes of the lines are the starting metabolism rate (SMR, % $O_2.h^{-1}.g^{-1}$) and oxygen metabolism rate (OMR, % $O_2.h^{-1}.g^{-1}$).

exhibited faster rates of consumption, with R50 values of 25.54 and 19.63 h, respectively (Figure 6).

Respiratory activity is analogous to the germination time course. The RGT values estimated were 61.35, 46.50, and 36.25 h for GS, YS, and CS, respectively. The same trend was observed in the germination (%) and AL (cm) determined at 90 h, which were 11.1% and 0.59 cm (GS), 65.4% and 1.04 cm (YS), and 83.3% and 1.44 cm (CS). Seed maturation is essential for producing viable and germinable soybean seeds. The results demonstrate that GS shows delayed oxygen consumption and germination,

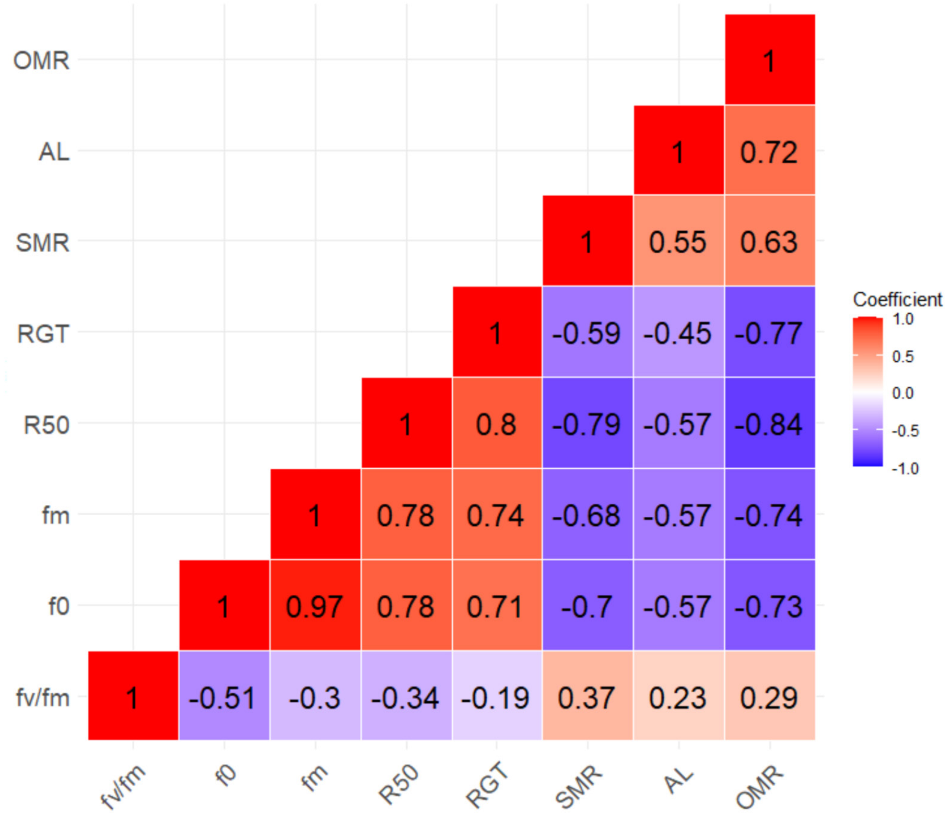

FIGURE 7 | Correlation coefficients of chlorophyll fluorescence minimum (f0) and maximum (fm), photosynthetic activity (fv/fm), starting metabolism rate (SMR, % $O_2.h^{-1}.g^{-1}$), oxygen metabolism rate (OMR, % $O_2.h^{-1}.g^{-1}$), time for seeds to reach 50% O_2 (R50, h), relative germination time (RGT), and axis length (AL, cm) for control, yellow, and green seeds. The variables are ordered based on the correlation coefficient's magnitude.

suggesting that Chl retention may negatively impact respiratory efficiency and germination potential.

3.6 | Associations Between Chl Fluorescence, Oxygen Consumption Rates, and Germination of Soybean Seeds Produced Under HDS

Examining the Pearson correlation coefficients of the results, a strong negative correlation can be seen between the Chl fluorescence (f0 and fm) of the seeds with the variables SMR, OMR, and AL (Figure 7). In addition, f0 and fm were positively correlated with R50 and RGT. However, the correlation between fv/fm and the variables mentioned above was not found to be significant (Figure 7); it was not considered suitable for inclusion in the PCA. In a second step, a PCA was carried out to explore the data in a two-dimensional space, maintaining as much information as possible. This analysis indicated that the first two components (PC1 and PC2) accounted for a high proportion of the variance (83.9%). The correlation matrix showed moderate coefficients for several of the variables. The correlations were analyzed by the angles between the vectors of the variables in the biplot graph of PC1 and PC2, where acute angles indicate positive correlations, and obtuse angles correspond to negative correlations between the variables. In PC1, it could be observed that high f0 and fm values were positively associated with R50 and RGT (Figure 8). Also, it can be seen that the vectors of the above variables were oriented towards GS, which would indicate

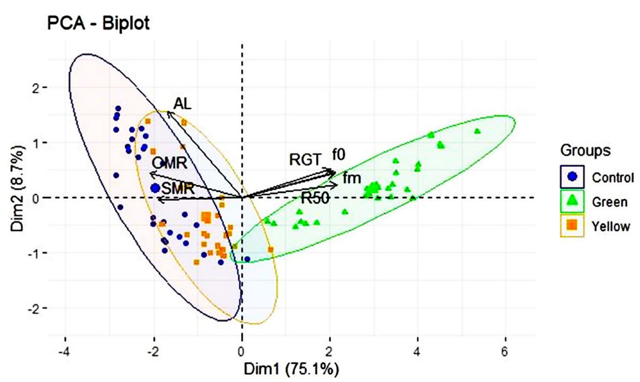

FIGURE 8 | Biplot of the first and second principal components of principal components analysis showing relationships between chlorophyll fluorescence minimum (f0) and maximum (fm); starting metabolism rate (SMR, % $O_2.h^{-1}.g^{-1}$); oxygen metabolism rate (OMR, % $O_2.h^{-1}.g^{-1}$); time to reach 50% O_2 (R50, h); relative germination time (RGT, h); and axis length (AL, cm) at 90 h of incubation of control, yellow, and green seeds.

that high levels of Chl fluorescence would be associated with seeds that require more time to initiate the germination process, as evidenced by the R50 and RGT variables (Figure 8). Meanwhile, for PC2, a negative association was found for the SMR variable and a positive association for OMR and AL. This would suggest that seeds with higher Chl fluorescence intensity would have a lower initial metabolic rate and take longer to germinate.

The Chl content of the GS obtained in this experiment had a significant impact on its viability and vigor compared to the CS and YS. Similarly, in the experiment of O_2 consumption during imbibition, very low germination was observed. It can be hypothesized that GS metabolic rates are associated with low physiological quality. However, it is also possible that other physiological conditions, such as alterations in the normal seed development resulting in embryonic dormancy, may also be involved, being important to study those other possible causes of germination failures in GS.

4 | Final Considerations

- HDS-matured GS show much lower quality than YS matured under the same conditions. YS matured under HDS conditions resembles seeds matured under nonstress conditions (NS).
- HDS conditions affected Chl degradation, resulting in GSs with lower viability and vigor.
- Higher Chl fluorescence levels and lower oxygen consumption rates, as well as reduced percentage and germination rates, were detected in the GSs.
- The initial metabolic rate and oxygen consumption rate were found to be negatively correlated with Chl fluorescence intensity and positively correlated with embryonic AL.
- Time to reach 50% of oxygen consumption was positively associated with Chl fluorescence intensity and negatively associated with embryonic AL.

Author Contributions

M.A.M., B.v.D., and B.O. performed the research. M.A.M. and B.v.D. analyzed the data. M.A.M., B.v.D., B.O., N.H.M., and C.O.G. wrote the paper. N.H.M. and C.O.G. designed the research.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer Review

The peer review history for this article is available in the Supporting Information for this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1** Peer review. **Figure S1.** Germination time course of green (GS) and yellow (YS) seeds produced in the field in 2017/2018 season, for soybean cultivars SRM 3410, SPS 4X4, and DM 4214 STS. Bars represent the mean values ± standard deviations of three replicates of 10 seeds. The dashed line corresponds to 50% germination. **Figure S2.** Germination time course of green (GS), yellow (YS), and control (CS) seeds produced in the greenhouse, for soybean cultivars SRM 3410, SPS 4X4, and DM 4214 STS. Bars represent the mean values ± standard deviations of three replicates of 10 seeds. The dashed line corresponds to 50% germination.