Engenharia Agrícola

ISSN: 1809-4430 (online)

www.engenhariaagricola.org.br | www.scielo.br/j/eagri

Scientific Paper

DOI: http://dx.doi.org/10.1590/1809-4430-Eng.Agric.v45e20250011/2025

PHYSICAL, PHYSIOLOGICAL, AND NUTRITIONAL CHARACTERIZATION OF SOYBEAN SEEDS AND CANONICAL INTERRELATIONS

Gabriel M. W. Bruinsma[1], Ivan R. Carvalho[1*], Leonardo C. Pradebon[2], Amauri De Carli Alchieri[3], Murilo V. Loro[2], Willyan J. A. Bandeira[1], João P. Dalla Roza[1], Ivandro Reolon[2]

[1*]Corresponding author. Postgraduate Program in Environmental Systems and Sustainability, Regional University of the Northwest of the State of Rio Grande do Sul (UNIJUÍ)/Íjui - RS, Brazil.
E-mail: ivan.carvalho@unijui.edu.br | ORCID ID: https://orcid.org/0000-0001-7947-4900

KEYWORDS

dicamba, application timing, diameter, circumference, germination, phenomics.

ABSTRACT

Technological advances in soybean (*Glycine max* L.) cultivation, particularly with *Xtend* technology cultivars, require appropriate and conscious management practices. This study aimed to identify canonical interrelationships among the physical, physiological, and nutritional parameters of soybean seeds carrying the *DMO* gene, using dicamba applied in both pre- and post-emergence stages. The experiment was conducted in Augusto Pestana, RS, Brazil, during the 2022/2023 growing season. Treatments consisted of eight dicamba application timings (pre- and post-emergence) across five Xtend cultivars. After harvesting, seed physical traits (diameter, circumference, perimeter, and area), germination, seedling viability, and seed nutritional components were evaluated. Seed diameter, perimeter, and area varied among cultivars, and reductions in these traits were associated with lower nutritional quality. Dicamba applications during the vegetative stages caused less damage to the physiological and nutraceutical quality of the seeds.

INTRODUCTION

The introduction of new technological packages for soybean (*Glycine max* L.) cultivation has enabled the use of different weed control strategies, including auxin herbicides, enhancing the control of difficult-to-control species (Solomon & Bradley, 2014). Among the genetic modifications, transgenic *Xtend* cultivars containing the *Dicamba Monooxygenase* (DMO) gene, conferring tolerance to the herbicide dicamba, are particularly notable (Dan et al., 2010). Seed production management, especially in the field, must maintain high physiological and biological quality to preserve these and other technologies transmitted through seeds, ensuring vigor, uniformity, and performance in the resulting plants (França-Neto et al., 2016).

The physical characteristics of soybean seeds, particularly diameter and circumference uniformity, contribute to sowing precision and promote homogeneous seed distribution in the sowing furrow (Pádua et al., 2010). Larger seeds contain greater reserves, which support seedling emergence in the field (Carvalho & Nakagawa, 2012; Bianchi et al., 2022). The chemical composition of soybean seeds, particularly oil and protein content, influences physiological quality and is genetically determined. However, it may be altered during the crop cycle due to environmental factors or management practices, especially during seed filling (Delarmelino-Ferraresi et al., 2014).

Seed quality reduction, or deterioration, may result from stress during harvest, processing, and storage, as well as from in-field conditions. These include excessive or insufficient moisture, pests and diseases, nutritional management, or herbicides. Auxin hormones can affect soybean seeds by deregulating gibberellin biosynthesis and increasing abscisic acid concentrations, leading to dormancy and reduced germination due to delayed root

[1] Postgraduate Program in Environmental Systems and Sustainability, Regional University of the Northwest of the State of Rio Grande do Sul (UNIJUÍ)/Íjui - RS, Brazil.
[2] Center for Rural Sciences, Department of Plant Science, Federal University of Santa Maria (UFSM)/Santa Maria - RS, Brazil.
[3] Center for Agricultural Sciences at the Northwest University of the State of Rio Grande do Sul (UNIJUÍ)/Íjui - RS, Brazil.

Area Editor: Gizele Ingrid Gadotti
Received in: 1-16-2025
Accepted in: 6-17-2025

protrusion through the seed coat (Shuai et al., 2017). Ceretta et al. (2023) reported reductions of 62.3% and 63% in the vigor and germination of soybean seeds, respectively, following dicamba application combined with the pre-emergence herbicides imazetapyr/flumioxazin before sowing.

The relationship among physiological, nutritional, and physical characteristics of soybean seeds, along with potential effects caused by specific management practices applied in the seed production field, can be revealed and interpreted through canonical correlation analysis, a method that allows investigation of interrelationships between groups of characters (Carvalho et al., 2015; Cruz et al., 2012). Given the increasing cultivation of *Xtend* soybean cultivars, this study aimed to identify canonical correlations between physical, physiological, and nutritional parameters of soybean seeds carrying the *DMO* gene, following dicamba applications in pre- and post-emergence stages.

MATERIAL AND METHODS

The experiment was conducted in the experimental area of the Escola Fazenda of the Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ), located in Augusto Pestana, RS, Brazil (28°00′14″ S, 52°00′22″ W, 328 m elevation). The soil in the area is classified as a Typical Dystrophic Red Oxisol, and the climate is classified as *Cfa* (humid subtropical), according to Köppen.

The experimental design was a randomized complete block design, arranged in a factorial scheme with eight dicamba application timings × five soybean cultivars with Xtend technology (Table 1), totaling 40 treatments. The blocks were used in the evaluations performed on the seeds harvested from each treatment. Herbicide applications were conducted under favorable conditions, which consisted of a relative humidity above 60%, air temperature below 30 °C, and wind speed under 10 km h^{-1}, using equipment set for a spray solution volume of 150 L ha^{-1}.

TABLE 1. Treatments, application timings, number of applications, and commercial product dose for each application.

Treatment	Time of application	Number of applications	Xtendicam + Xtend Protect (L ha^{-1})
AB	No molecule application	0	0
PS	Pre-sowing	1	1.0; 1.0
PS+V4	Pre-sowing + V4	2	1.0; 1.0
PS+R2	Pre-sowing + R2	2	1.0; 1.0
PS+R3	Pre-sowing + R3	2	1.0; 1.0
V4	V4	1	1.0; 1.0
R2	R2	1	1.0; 1.0
R3	R3	1	1.0; 1.0

V4: four fully developed leaves; R2: full flowering; R3: beginning of pod development.

Each experimental unit covered 22 m^2, with 14 sowing rows spaced 0.5 meters apart. Sowing was performed on December 21, 2022, with a sowing density of 16 seeds m^{-1}. Base fertilization consisted of 250 kg ha^{-1} of the 05-35-12 (N-P-K) formulation. The used *Xtend* cultivars were BMX TORQUE 57ix60 i2x, M5710 i2x, FT4426 i2x, BMX NEXUS 64ix66 i2x, and FT4664 i2x. Phytosanitary management was conducted preventively to minimize biotic interference in the experimental results.

The plants from the 10 central rows of each experimental unit were harvested mechanically at the R8 stage (full maturity). The seeds were then manually cleaned and dried in a forced-air circulation oven at 25 °C until they reached 13% moisture. A subsample of 25 seeds per block was taken from each treatment for physical analysis, and the following seed parameters were measured: seed diameter (S_DIA, pixels), seed perimeter (S_PERI, pixels), seed area (S_AREA, pixels), and seed circularity (S_CIRCULARITY, pixels), using phenomic analysis via the *R pliman* software (Olivoto, 2022).

Subsequently, the physiological seed quality was evaluated at the Laboratory of Seeds of the Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ) immediately after harvest, without accelerated aging, using eight samples (blocks) of 25 seeds each from each treatment. The following parameters were assessed:

I) Germination – Seeds were placed on Germitest-type germination paper moistened with distilled water at 2.5 times the paper's dry mass. The seeds were evenly distributed, and the papers were folded and rolled before being incubated in a room set to 25 °C under constant light, with periodic rehydration to compensate for moisture loss. The first count (FC_V, %), consisting of the number of germinated seeds, was recorded on the 5th day, based on radicle emergence. The number of normal seedlings (NS, %), abnormal seedlings (AS, %), and dead seeds (DS, %) was determined on the 8th day, according to Brazil (2009).

II) Seedling length (SL, cm) – After the second germination assessment, five normal seedlings were randomly selected from each of the eight replicates. The length was measured from the collar (hypocotyl–radicle transition) to the tip of the seedling.

The chemical composition of the seeds from each treatment was analyzed using near-infrared spectroscopy (NIRS). The following parameters were measured: protein (PTN, %), oil (OL, %), crude fiber (CB, %), mineral matter

(MM, %), palmitic acid (PALMITIC_AG, %), stearic acid (STEARIC_AG, %), oleic acid (OLEIC_AG, %), linoleic acid (LINOLEIC_AG, %), and linolenic acid (LINOLENIC_AG, %).

Subsequently, the data were tested for normality of errors using the Shapiro-Wilk test and for homogeneity of residual variances using Bartlett's test. Then, analysis of variance at a 5% significance level was performed using the F-test, testing for the interaction between cultivars and treatments. Multivariate canonical correlation analysis was used to evaluate the relationships among the physiological, physical, and nutritional seed traits. Additionally, a biplot analysis of principal components was conducted to identify which treatments were most influenced by specific attributes. All statistical analyses were performed using *R software* (R Core Team, 2023).

RESULTS AND DISCUSSION

The analysis of variance (Table 2) revealed significant effects of the cultivar factor on all measured variables. Seedling length, seed area, seed perimeter, and seed diameter showed significance in the treatment factor. The cultivar x treatment interaction was significant at 5% probability by the F-test for normal seedlings, first count, seed area, seed perimeter, and seed diameter. The coefficients of variation ranged from low to high across the evaluated variables.

TABLE 2. Joint analysis of variance for physiological and physical seed variables: seedling length (SL, cm), normal seedlings (NS, %), first count (FC, %), seed area (SA, pixels), seed perimeter (SP, pixels), and seed diameter (SD, pixels).

Variation Favor	DF	MS		
		SL	**NS**	**FC**
Cultivar (C)	4	14.34*	1641.88*	15.70*
Treatment (T)	7	3.90*	14.39	5.25
Block	7	0.91	38.74	8.34
C x T	28	1.08	54.73*	10.50*
Residual	273	1.21	32.95	5.61
CV (%)		11.08	6.63	2.40
Variation Favor	**DF**	**MS**		
		SA	**PS**	**SD**
Cultivar (C)	4	96704.79*	634.55*	60.86*
Treatment (T)	7	3584.91*	32.41*	1.82*
Block	24	80021.15*	509.25*	48.19*
C x T	28	5195.49*	37.89*	3.18*
Residual	936	764.62	7.61	0.46
CV (%)		5.31	3.24	2.67

Significant at 5% probability according to the F-test. (DF): degrees of freedom; (MS): mean square; (CV): coefficient of variation.

The interaction between cultivars and treatments for seed physical quality (Table 3) showed that seed diameter was greater in the cultivars BMX NEXUS, BMX TORQUE, and M5710, and smaller in FT4426 and FT4664. The absence of dicamba application (AB) resulted in a smaller seed diameter compared to all other treatments in the BMX NEXUS and BMX TORQUE cultivars. Applications at the PS+R2 and R3 stages and PS, PS+V4, V4 and R2 in the FT4426 and M5710, respectively, reduced seed diameter relative to the AB treatment. Miller & Norsworthy (2018) observed negative effects on soybean seed quality when dicamba was applied during the reproductive stage. Similarly, Silva et al. (2018) reported that auxin herbicides reduced soybean seed quality regardless of the stage of application, findings that support those observed in this study.

The BMX NEXUS and BMX TORQUE cultivars exhibited a greater seed perimeter compared to the other cultivars. In both cases, the AB treatment showed the lowest values relative to all dicamba application treatments. BMX NEXUS and FT4426 showed a larger range in perimeter values among treatments, whereas FT4664 exhibited similarity of pixels for all treatments. Similarly, the highest values for seed area were found in BMX NEXUS and BMX TORQUE, with all dicamba application treatments outperforming the AB treatment in both cultivars. FT4426 showed greater heterogeneity in seed area among treatments and exhibited the lowest values overall. In contrast, FT4664 demonstrated the least variation in seed area among treatments.

TABLE 3. Interaction between cultivars and treatments for the variables seed diameter (pixels), seed perimeter (pixels), and seed area (pixels).

Cultivar	AB	PS	PS+R2	PS+R3	PS+V4	R2	R3	V4
				Seed diameter (pixels)				
BMX NEXUS	25 bC	26 aA	26 bB	26 aB	27 aA	26 aB	26 aA	26 aA
BMX TORQUE	25 bB	26 aA	26 aA	26 aA	26 bA	25 bB	26 bA	26 bA
FT 4426	25 cB	25 bB	24 dC	25 bA	25 dB	25 bA	24 dC	25 cA
FT 4664	25 cA	25 bA	25 cA	25 bA	25 cA	25 bA	25 cA	25 dA
M 5710	26 aA	25 bB	26 aA	26 aA	25 cB	25 bB	26 bA	25 cB
				Seed perimeter (pixels)				
BMX NEXUS	84 aC	88 aA	86 aB	86 aB	89 aA	87 aB	88 aA	89 aA
BMX TORQUE	85 aB	87 aA	87 aA	87 aA	87 bA	85 bB	86 bA	86 bA
FT 4426	83 bB	83 bB	81 cC	84 bA	83 dB	85 bA	81 dC	84 cA
FT 4664	84 aA	83 bA	83 bA	83 bA	84 cA	83 bA	83 cA	83 dA
M 5710	85 aB	84 bB	87 aA	86 aB	85 cB	85 bB	87 bA	85 cB
				Seed area (pixels)				
BMX NEXUS	514 aC	522 aA	528 bB	531 aB	568 aA	514 aB	559 aA	567 aA
BMX TORQUE	520 aB	546 aA	548 aA	537 aA	545 bA	518 bB	538 bA	540 bA
FT 4426	493 bB	498 bB	469 dC	508 bA	492 dB	514 bA	471 dC	513 cA
FT 4664	506 bA	502 bA	498 cA	494 bA	513 cA	504 bA	500 cA	493 dA
M 5710	529 aB	511 bB	542 aA	531 aB	526 cB	520 bB	540 bA	526 cB

AB: absence of dicamba application; PS: application at pre-sowing; PS+R2: application at pre-sowing + R2; PS+R3: application at pre-sowing + R3; PS+V4: application at pre-sowing + V4; R2: application at R2; R3: application at R3; V4: application at V4.

Significant interactions between cultivars and treatments were observed in the analysis of seed physiological quality (Table 4). The lowest first count values were recorded in treatments AB, PS+V4, and V4, all with 96% in the BMX NEXUS cultivar. The other cultivars showed higher percentages of first count, reaching above 97% in BMX TORQUE and above 98% in FT4426, FT4664, and M5710. The cultivar FT4664 stood out with 100% germination in the AB, V4, R2, PS+R2, and R3 treatments. For the second count, which reflects the percentage of normal seedlings, BMX TORQUE showed the lowest percentages among all cultivars: 75%, 77% and 78% in the PS, PS+R3, and PS+R2 treatments, respectively, all lower than the AB treatment (80%). These effects may be attributed to hormonal imbalances caused by the herbicides. Shuai et al. (2017) reported that exogenous auxin applications negatively affected gibberellic acid biosynthesis and promoted soybean seed dormancy, in addition to inhibiting germination by delaying radicle protrusion and weakening seed coat rupture. In the FT4664 cultivar, the AB treatment resulted in a higher proportion of normal seedlings (92%) compared to treatments with applications. However, the PS+R2 treatment showed the highest percentage of normal seedlings (94%).

TABLE 4. Interaction between cultivars and treatments for the variables first count (%) and normal seedlings (%).

Cultivar	AB	PS	PS+R2	PS+R3	PS+V4	R2	R3	V4
				First count (%)				
BMX NEXUS	96 bB	100 aA	100 aA	100 aA	96 bB	98 aA	99 aA	96 bB
BMX TORQUE	99 aA	98 aA	97 aA	98 aA	100 aA	100 aA	98 aA	100 aA
FT 4426	100 aA	98 aA	98 aA	99 aA	98 bA	100 aA	100 aA	99 aA
FT 4664	100 aA	99 aA	100 aA	98 aA	99 aA	100 aA	100 aA	100 aA
M 5710	100 aA	100 aA	98 aA	99 aA	98 aA	100 aA	98 aA	99 aA
				Normal seedlings (%)				
BMX NEXUS	82 bB	84 bB	90 aA	84 bB	82 bB	82 bB	82 bB	83 bB
BMX TORQUE	80 bA	75 cB	78 cB	77 cB	82 bA	80 bA	84 bA	78 bB
FT 4426	90 aA	92 aA	94 aA	92 aA	92 aA	90 aA	90 aA	90 aA
FT 4664	92 aA	90 aA	84 bA	88 aA	88 aA	90 aA	84 bA	90 aA
M 5710	89 aA	90 aA	89 aA	90 aA	92 aA	93 aA	92 aA	88 aA

AB: absence of dicamba application; PS: application at pre-sowing; PS+R2: application at pre-sowing + R2; PS+R3: application at pre-sowing + R3; PS+V4: application at pre-sowing + V4; R2: application at R2; R3: application at R3; V4: application at V4.

The analysis of the effects of the cultivars (Table 5) on average seedling length showed that the FT4664, M5710, and BMX NEXUS cultivars exhibited greater seedling lengths, while lower performance was observed in BMX TORQUE and FT4426. Regarding the effect of treatments on average seedling length, only the treatment with application at the R3 stage showed a superior response.

TABLE 5. Effects of cultivars and treatments on seedling length (cm).

Treatment							
R3	V4	PS+R3	R2	PS+R2	PS	PS+V4	AB
10.55 a	10.07 b	10.01 b	9.98 b	9.90 b	9.81 b	9.81 b	9.45 b

Cultivar	
FT 4664	10.43 a
M 5710	10.24 a
BMX NEXUS	10.14 a
FT 4426	9.64 b
BMX TORQUE	9.28 b

AB: absence of dicamba application; PS: application at pre-sowing; PS+R2: application at pre-sowing + R2; PS+R3: application at pre-sowing + R3; PS+V4: application at pre-sowing + V4; R2: application at R2; R3: application at R3; V4: application at V4.

The Dunnett test (Table 6) was applied to compare the PS, PS+R2, PS+R3, PS+V4, R2, R3, and V4 treatments with the AB treatment for the nutritional seed traits. Significant effects were observed only for palmitic acid, with reductions of −1.57%, −1.07%, and −1.06% in the PS, V4, and PS+R3 treatments, respectively. The other nutritional components—protein, oil, crude fiber, mineral material, stearic acid, oleic acid, linoleic acid, and linolenic acid—showed no significant changes in the treatments compared to the absence of dicamba application. The reduction in palmitic acid in soybean seeds is a desirable trait in breeding programs, as it is beneficial for human nutrition. In fact, excessive consumption of this fatty acid has been associated with cardiovascular and prostate-related diseases (Xue et al., 2022; Sangiovo et al., 2023). According to Taylor et al. (2017), a comparison of dicamba-tolerant cultivars and conventional cultivars revealed only small differences in nutritional composition. However, the nutritional values of both were consistent with the crop composition data for conventional soybean cultivars available in the International Life Sciences Institute Crop Composition Database (ILSI-CCDB).

TABLE 6. Dunnett's test comparing the PS, PS+R2, PS+R3, PS+V4, R2, R3, and V4 treatments with the AB treatment for the seed nutritional quality variables protein, oil, crude fiber, mineral matter, palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid.

Treatment	C-value	Estimate	Lwr-CI	Upr-CI	t value	p-value	sig
				PROTEIN			
PS	33.48	0.224	-0.598	1.046	0.666	0.977	ns
PS+R2	33.13	-0.124	-0.946	0.698	-0.369	0.999	ns
PS+R3	33.61	0.352	-0.470	1.174	1.047	0.827	ns
PS+V4	33.22	-0.030	-0.852	0.792	-0.089	1.000	ns
R2	33.12	-0.130	-0.952	0.692	-0.387	0.999	ns
R3	33.48	0.222	-0.600	1.044	0.660	0.978	ns
V4	33.30	0.048	-0.774	0.870	0.143	1.000	ns
				OIL			
PS	20.84	-0.074	-1.131	0.983	-0.171	1.000	ns
PS+R2	21.02	0.100	-0.957	1.157	0.231	1.000	ns
PS+R3	21.00	0.082	-0.975	1.139	0.190	1.000	ns
PS+V4	20.89	-0.028	-1.085	1.029	-0.065	1.000	ns
R2	20.84	-0.080	-1.137	0.977	-0.185	1.000	ns
R3	20,69	-0.224	-1.281	0.833	-0.518	0.994	ns
V4	20.79	-0.124	-1.181	0.933	-0.287	0.999	ns
				CRUDE FIBER			
PS	7.30	-0.012	-0.267	0.243	-0.115	1.000	ns
PS+R2	7.20	-0.110	-0.363	0.145	-1.055	0.823	ns
PS+R3	7.20	-0.108	-0.363	0.147	-1.036	0.834	ns
PS+V4	7.23	-0.084	-0.339	0.171	-0.806	0.941	ns
R2	7.25	-0.060	-0.315	0.195	-0.576	0.990	ns
R3	7.28	-0.032	-0.287	0.223	-0.307	0.999	ns
V4	7.32	0.010	-0.245	0.265	0.096	1.000	ns

MINERAL MATTER							
PS	5.65	0.002	-0.056	0.060	0.085	1.000	ns
PS+R2	5.65	-0.002	-0.060	0.056	-0.085	1.000	ns
PS+R3	5.66	0.012	-0.046	0.070	0.510	0.995	ns
PS+V4	5.64	-0.006	-0.064	0.052	-0.255	0.999	ns
R2	5.65	0.002	-0.056	0.060	0.085	1.000	ns
R3	5.68	0.032	-0.026	0.090	1.358	0.617	ns
V4	5.65	-0.002	-0.060	0.056	-0.085	1.000	ns
PALMITIC ACID							
PS	10.59	-1.572	-2.613	-0.531	-3.693	0.005	*
PS+R2	11.57	-0.598	-1.639	0.443	-1.405	0.585	ns
PS+R3	11.10	-1.064	-2.105	-0.023	-2.500	0.089	*
PS+V4	12.02	-0.140	-1.181	0.901	-0.329	0.999	ns
R2	11.68	-0.488	-1.529	0.553	-1.147	0.765	ns
R3	12.39	0.226	-0.815	1.267	0.531	0.994	ns
V4	11.09	-1.070	-2.111	-0.029	-2.514	0.086	*
STEARIC ACID							
PS	4.45	-0.080	-0.339	0.179	-0.757	0.956	ns
PS+R2	4.56	0.022	-0.237	0.281	0.208	1.000	ns
PS+R3	4.40	-0.136	-0.395	0.123	-1.286	0.669	ns
PS+V4	4.46	-0.074	-0.333	0.185	-0.700	0.970	ns
R2	4.36	-0.170	-0.429	0.089	-1.608	0.447	ns
R3	4.52	-0.012	-0.271	0.247	-0.114	1.000	ns
V4	4.43	-0.108	-0.367	0.151	-1.021	0.843	ns
OLEIC ACID							
PS	25.71	2.146	-1.335	5.627	1.508	0.513	ns
PS+R2	24.21	0.640	-2.841	4.121	0.450	0.998	ns
PS+R3	24.72	1.156	-2.325	4.637	0.812	0.939	ns
PS+V4	22.96	-0.610	-4.091	2.871	-0.423	0.998	ns
R2	23.64	0.070	-3.411	3.551	0.049	1.000	ns
R3	23.82	0.254	-3.227	3.735	0.178	1.000	ns
V4	24.53	0.960	-2.521	4.441	0.675	0.976	ns
LINOLEIC ACID							
PS	55.68	0.914	-1.869	3.697	0.803	0.942	ns
PS+R2	55.35	0.590	-2.193	3.373	0.518	0.994	ns
PS+R3	56.09	1.326	-1.457	4.109	1.165	0.752	ns
PS+V4	56.33	1.568	-1.215	4.351	1.378	0.603	ns
R2	57.02	2.256	-0.527	5.039	1.982	0.245	ns
R3	54.67	-0.094	-2.877	2.689	-0.083	1.000	ns
V4	56.43	1.668	-1.115	4.451	1.466	0.542	ns
LINOLENIC ACID							
PS	3.67	-1.688	-3.942	0.566	-1.831	0.317	ns
PS+R2	4.77	-0.588	-2.842	1.666	-0.638	0.982	ns
PS+R3	3.79	-1.564	-3.818	0.690	-1.697	0.392	ns
PS+V4	3.80	-1.562	-3.816	0.692	-1.695	0.393	ns
R2	3.43	-1.930	-4.184	0.324	-2.094	0.200	ns
R3	4.81	-0.548	-2.802	1.706	-0.595	0.988	ns
V4	3.77	-1.588	-3.842	0.666	-1.723	0.376	ns

AB: absence of dicamba application; PS: application at pre-sowing; PS+R2: application at pre-sowing + R2; PS+R3: application at pre-sowing + R3; PS+V4: application at pre-sowing + V4; R2: application at R2; R3: application at R3; V4: application at V4.
(C-value: average value of cultivars for treatment); (lwr-CI: lower confidence interval); (upr-CI: upper confidence interval); (t-value: calculated value); (p-value: probability); (sig: significance); (ns: not significant); (*: significant).

According to the canonical loadings obtained from the comparative analysis of the physical and nutritional seed traits (Table 7), the first canonical pair revealed a correlation of r = 0.755 between groups, indicating that reductions in seed area, perimeter, and diameter were associated with decreases in total protein, mineral matter, and linolenic acid levels, and with increases in palmitic and stearic acids. In contrast, the second canonical pair showed a positive intergroup correlation of r = 0.692 between circularity and the contents of total protein, crude fiber, and linoleic acid. A correlation of r = 0.860 was observed in the first canonical pair between physiological and nutritional seed traits, indicating strong interdependence between these trait groups (Carvalho et al., 2015). An increase in abnormal seedlings—accompanied by a consequent reduction in normal seedlings—was related to reductions in total protein, crude fiber, and linoleic acid, and increases in oil content and stearic and oleic acids.

TABLE 7. Canonical loadings of physical, physiological, and nutritional seed trait groups in five *Xtend* soybean cultivars managed with dicamba applications in pre- and post-emergence stages.

Trait	Canonical pair		Trait	Canonical pair
	1st	2nd		1st
PHYSICS			PHYSIOLOGICAL	
S_AREA	-0.251	-0.024	FC_V	-0.177
S_PERI	-0.252	-0.072	NS	-0.981
S_DIA	-0.292	-0.030	DS	0.359
S_CIRCULARITY	0.164	0.908	AS	0.995
NUTRITIONAL			NUTRITIONAL	
PTN	-0.472	0.497	PTN	-0.408
OL	-0.063	0.012	OL	0.888
CB	-0.092	0.442	CB	-0.601
MM	-0.353	0.228	MM	0.189
PALMITIC_FA	0.290	-0.161	PALMITIC_FA	0.181
STEARIC_FA	0.277	0.091	STEARIC_FA	0.551
OLEIC_FA	0.110	-0.075	OLEIC_FA	0.567
LINOLEIC_FA	-0.037	0.251	LINOLEIC_FA	-0.782
LINOLENIC_FA	-0.424	-0.194	LINOLENIC_FA	-0.087
R	0.755	0.692	R	0.860
p	0.003	0.045	P	0.000

Seed diameter (S_DIA); seed perimeter (S_PERI); seed area (AREA_S); seed circularity (S_CIRCULARITY); first count (FC_V); normal seedlings (NS); abnormal seedlings (AS); dead seeds (DS); protein (PTN); oil (OL); crude fiber (CB); mineral matter (MM); palmitic acid (PALMITIC_FA); stearic acid (STEARIC_FA); oleic acid (OLEIC_FA); linoleic acid (LINOLEIC_FA); linolenic acid (LINOLENIC_FA).

Regarding the contribution to variability (Figure 1), seed circularity, linolenic acid, and abnormal seedlings showed the greatest variability, each contributing more than 9%, indicating a high coefficient of variation due to cultivar and environmental effects. Similarly, seed area, perimeter, and diameter showed contributions close to 8%. On the other hand, oil and crude fiber contents exhibited lower variability, around 2%, suggesting more homogeneous values across cultivars and treatments. Also notable was the low variability in first count, palmitic acid, oleic acid, dead seeds, and mineral matter, with contributions close to 4%.

The principal component analysis (Figure 1 – biplot) showed that the R2 treatment had greater influence on the physical seed traits diameter, area, circumference, and linoleic acid. The PS+R2 treatment was associated with the percentage of dead seeds, normal seedlings, seed oil, and palmitic acid. Both treatments at the R2 stage affected nearly half of the evaluated traits. The PS treatment had a strong influence on oleic acid and total protein contents, as well as on the percentage of abnormal seedlings and first count. The use of dicamba increases electrical conductivity, resulting in the leaching of sugars, amino acids, electrolytes, and other water-soluble compounds, ultimately leading to reduced seed vigor (Costa et al., 2020).

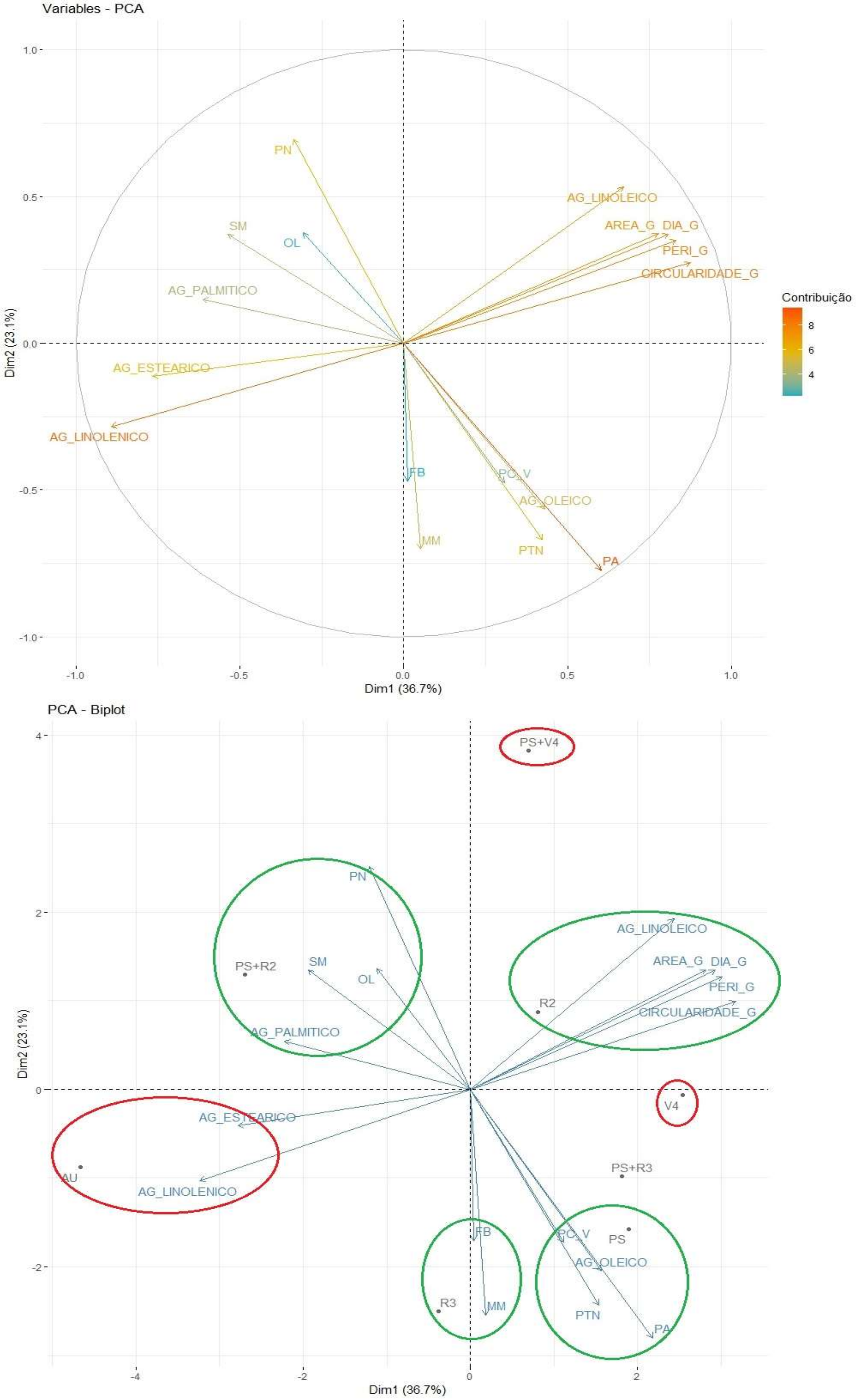

FIGURE 1. Biplot of the principal components for the following variables: seed diameter (S_DIA), seed perimeter (S_PERI), seed area (S_AREA), seed circularity (S_CIRCULARITY), first count (FC_V), normal seedlings (NS), abnormal seedlings (AS), dead seeds (DS), protein (PTN), oil (OL), crude fiber (CB), mineral material (MM), palmitic acid (PALMITIC_FA), stearic acid (STEARIC_FA), oleic acid (OLEIC_FA), linoleic acid (LINOLEIC_FA), and linolenic acid (LINOLENIC_FA). Treatments: AB: absence of dicamba application; PS: application at pre-sowing; PS+R2: application at pre-sowing + R2; PS+R3: application at pre-sowing + R3; PS+V4: application at pre-sowing + V4; R2: application at R2; R3: application at R3; V4: application at V4.

Seed MM and CB contents were affected only by the application at the R3 stage. In contrast, the AB treatment showed a weak influence on the levels of linolenic and stearic acids, indicating that these components were not altered by dicamba application in the *Xtend* soybean crop. The V4 and PS+V4 treatments stood out in the multivariate analysis (biplot), as they did not influence the physiological, physical, or nutritional seed traits, indicating that dicamba applications during the vegetative stage of *Xtend* soybeans have minimal impact.

CONCLUSIONS

Seed diameter, area, and perimeter in the BMX TORQUE and BMX NEXUS cultivars were greater in all treatments with dicamba application. The reduction in physical traits was correlated with the low nutritional seed quality.

Only the BMX TORQUE cultivar showed percentages of normal seedlings lower than 80% under the PS, PS+R3, and PS+R2 treatments.

The concentration of palmitic acid was reduced in the PS, V4, and PS+R3 treatments.

Dicamba applications during the vegetative crop stages caused fewer physiological, physical, and nutritional changes in the seeds.

REFERENCES

Bianchi, M. C., Vilela, N. J. D., Carvalho, E. R., Pires, R. M. D. O., Santos, H. O. D., & Bruzi, A. T. (2022). Soybean seed size: How does it affect crop development and physiological seed quality? *Journal of Seed Science, 44*, e202244010. https://doi.org/10.1590/2317-1545v44255400

Brazil. Ministry of Agriculture, Livestock and Food Supply. Secretariat of Agricultural Defense. (2009). *Rules for seed analysis*. https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/arquivos-publicacoes-insumos/2946_regras_analise__sementes

Carvalho, N. M., & Nakagawa, J. (2012). *Seeds: Science, technology and production* (5th ed.). FUNEP.

Carvalho, I. R., Souza, V. Q. D., Nardino, M., Follmann, D. N., Schmidt, D., & Baretta, D. (2015). Canonical correlations between morphological characters and production components in dual-purpose wheat. *Brazilian Agricultural Research, 50*(8), 690–697. https://doi.org/10.1590/S0100-204X2015000800007

Ceretta, J. M., Albrecht, A. J. P., Albrecht, L. P., Silva, A. F. M., & Yokoyama, A. S. (2023). Can pre-and/or post-emergent herbicide application affect soybean seed quality? *Caatinga Journal, 36*(4), 740–747. https://doi.org/10.1590/1983-21252023v36n401rc

Costa, E. M., Zuchi, J., Ventura, M. V. A., Pereira, L. S., Caetano, G. B., & Jakelaitis, A. (2020). Simulated drift of dicamba: Effect on the physiological quality of soybean seeds. *Journal of Seed Science, 42*, e202042014. https://doi.org/10.1590/2317-1545v42224236

Cruz, C. D., Regazzi, A. J., & Carneiro, P. C. S. (2012). *Biometric models applied to genetic improvement* (2nd ed.). UFV Ed.

Dan, H. A., Dan, L. G. M., Barroso, A. L. L., Procópio, S. O., Oliveira Jr, R. S., Silva, A. G., Lima, M. D. B., & Feldkircher, C. (2010). Residual activity of herbicides used in soybean agriculture on grain sorghum crop succession. *Weed, 28*, 1087–1095. https://doi.org/10.1590/S0100-83582010000500016

Delarmelino-Ferraresi, L. M., Villela, F. A., & Aumonde, T. Z. (2014). Physiological performance and chemical composition of soybean seeds. *Brazilian Journal of Agricultural Sciences, 9*(1), 14–18. https://doi.org/10.5039/agraria.v9i1a2864

França-Neto, J. D. B., Krzyzanowski, F. C., Henning, A. A., De Pádua, G. P., Lorini, I., & Henning, F. A. (2016). *High quality soybean seed production technology*. Embrapa Soja.

Miller, M. R., & Norsworthy, J. K. (2018). Soybean sensitivity to florpyrauxifen-benzyl during reproductive growth and the impact on subsequent progeny. *Weed Technology, 32*(2), 135–140. https://doi.org/10.1017/wet.2017.108

Pádua, G. P. D., Zito, R. K., Arantes, N. E., & França Neto, J. D. B. (2010). Influence of seed size on physiological seed quality and soybean yield. *Revista Brasileira de Sementes, 32*, 9–16. https://doi.org/10.1590/S0101-31222010000300001

Olivoto, T. (2022). Lights, camera, pliman! An R package for plant image analysis. *Methods in Ecology and Evolution, 13*(4), 789–798. https://doi.org/10.1111/2041-210X.13803

R Core Team. (2023). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.r-project.org/

Sangiovo, J. P., Carvalho, I. R., Pradebon, L. C., Loro, M. V., Port, E. D., Scarton, V. D. B., & Foleto, E. E. (2023). Selection of soybean lines based on a nutraceutical ideotype. *Revista de Agricultura Neotropical, 10*(3), e7356. https://doi.org/10.32404/rean.v10i3.7356

Silva, D. R. O., Aguiar, A. C. M., Novello, B. D., Silva, A. A. A., & Basso, C. J. (2018). Drift of 2,4-D and dicamba applied to soybean at vegetative and reproductive growth stage. *Rural Science, 48*(8), e20180179. https://doi.org/10.1590/0103-8478cr20180179

Shuai, H., Meng, Y., Luo, X., Chen, F., Zhou, W., Dai, Y., Qi, Y., Du, J., Yang, F., Liu, J., Tang, W., & Shu, K. (2017). Exogenous auxin represses soybean seed germination through decreasing the gibberellin/abscisic acid (GA/ABA) ratio. *Scientific Reports, 7*(1), 12620. https://doi.org/10.1038/s41598-017-13093-w

Taylor, M. L., Bickel, A., Mannion, R., Bell, E., & Harrign, G. G. (2017). Dicamba-tolerant soybeans (*Glycine max* L.) MON 87708 and MON 87708 × MON 89788 are compositionally equivalent to conventional soybean. *Journal of Agricultural and Food Chemistry, 65*(36), 8037 - 8045. https://doi.org/10.1021/acs.jafc.7b03844

Xue, Y., Gao, H., Liu, X., Tang, X., Cao, D., Luan, X., & Qiu, L. (2022). QTL mapping of palmitic acid content using specific-locus amplified fragment sequencing (SLAF-Seq) genotyping in soybeans (*Glycine max* L.). *International Journal of Molecular Sciences, 23*(19), 11273. https://doi.org/10.3390/ijms231911273