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Article

Physiological quality and seed chemical composition of soybean seeds under different altitude

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ABSTRACT: Seed quality plays an important role in the production of soybean. The objective of this study was to quantify the effects of producing region on seed chemical composition (oil, protein, and fatty acid content) and physiological quality of soybean. Twenty-eight soybean cultivars were evaluated in 2017/18 and 2018/19 crop seasons, and field trials were performed in different environments classified as high (846–963 m above sea level [asl]) or low altitude (336–480 m asl). The evaluated traits were percentage germination, accelerated aging, germination test, emergence speed index, and seed chemical composition (oil and protein contents, fatty acid profile). A significant effect of cultivar × environment interactions on all evaluated traits was observed. High-altitude environments produce soybeans with a greater protein content, and low altitudes yielded seeds with elevated oil content. Higher protein-content seeds have greater physiological potential, and seeds with higher oil content are negatively associated with physiological potential. High-altitude environments maximized the physiological quality of seeds. Linking genetics to target populations of environments ensures seed quality and benefits the entire soybean production chain.

Key words: fatty acid, oil, protein, soybean.

INTRODUCTION

The physiological quality of seeds directly impacts the initial development of plants and plant yield components. Physiological quality may be defined as the ability to perform vital features, such as germination, vigor, and longevity, which are essential to ensure successful production (Ebone et al. 2020). Previous research has shown that seedlings with low seed vigor exhibit reduced emergence, resulting in decreased leaf area, low numbers of pods and, consequently, diminished soybean yield (Ebone et al. 2020; Feliceti et al. 2020).

The seed quality depends directly on the environmental conditions and seeds (postharvest) (Lamichaney and Maity 2021). The high variability of climatic conditions in subtropical and tropical regions makes the production of high seed quality difficult. In this sense, choosing the best locations for seed production can make a substantial difference. Usually, locations with lower temperatures and dry conditions during preharvest can improve seed quality (Oliveira et al. 2021).

The chemical composition of seeds is also affected by environmental conditions (Assefa et al. 2018; Silva et al. 2017). In general, low temperatures increase protein content, and higher temperatures result in high seed oil content (Alsajri et al. 2020; Nakagawa et al. 2020). Oil and protein contents are related to physiological potential. Protein is one of the energy sources for plant embryos and helps the establishment and development of seedlings under field conditions (Wei et al. 2020). Seed oil content is responsible for storing lipids as triacylglycerol, which serves as a primary carbon source and aids in respiration during the pregermination stage (Wendt et al. 2017).

The fatty acid composition of seeds is also affected by environmental conditions, mainly temperature, and is related to seed quality (Alsajri et al. 2020; Bellaloui et al. 2017a). Bellaloui et al. (2017a) reported a positive association between germination and palmitic acid levels, and a negative relationship between germination and linolenic and linoleic acids. Woyann et al. (2019) reported that soybean breeding has resulted in reduced levels of fatty acids, such as linoleic and linolenic acids, and increased abundance of oleic, stearic, and palmitic acids to improve the quality and stability of soybean oil used for biodiesel production. These changes in the chemical composition of seeds have contributed positively to physiological quality. In this context, the objective of this study was to quantify the effect of producing region on seed chemical composition (oil, protein, and fatty acids) and physiological quality of soybean seeds.

MATERIAL AND METHODS

Plant material and experimental design

In this study, 28 soybean cultivars available for cultivation in Southern Brazil were evaluated (Table 1). In the 2017/18 and 2018/19 cropping seasons, field trials were performed in different environments, classified as high (846–963 m above

Table 1. Soybean cultivars, year of release, maturity group (MG), technology, and plant breeding company.

Code	Cultivar	Year of release	MG	Technology	Plant breeding company
1	AS 3610 IPRO	2014	6.6	RR2	Bayer
2	AS 3730 IPRO	2014	7.3	RR2	Bayer
3	5855RSF IPRO	2016	5.5	RR2	GDM Seeds
4	63I64RSF IPRO	2018	6.3	RR2	GDM Seeds
5	68I70RSF IPRO	2016	6.8	RR2	GDM Seeds
6	58I60RSF IPRO	2018	5.8	RR2	GDM Seeds
7	7166RSF IPRO	2015	6.8	RR2	GDM Seeds
8	50I52RSF IPRO	2018	5.0	RR2	GDM Seeds
9	5958RSF IPRO	2015	5.8	RR2	GDM Seeds
10	M5705 IPRO	2015	5.7	RR2	Bayer
11	M5730 IPRO	2016	5.7	RR2	Bayer
12	M5838 IPRO	2017	5.8	RR2	Bayer
13	M5917 IPRO	2014	5.9	RR2	Bayer
14	M5947 IPRO	2015	6.3	RR2	Bayer
15	M6210 IPRO	2013	6.6	RR2	Bayer
16	M6410 IPRO	2013	6.6	RR2	Bayer
17	NA 5909 RG	2012	6.2	RR	Syngenta
18	NS 5445 IPRO	2013	5.5	RR2	Syngenta
19	NS 5959 IPRO	2013	5.9	RR2	Syngenta
20	NS 6006 IPRO	2016	5.7	RR2	Syngenta
21	NS 6601 IPRO	2018	6.6	RR2	Syngenta
22	NS 6828 IPRO	2017	6.4	RR2	Syngenta
23	NS 6906 IPRO	2016	6.4	RR2	Syngenta
24	NS 6909 IPRO	2013	6.2	RR2	Syngenta
25	NS 7300 IPRO	2014	7.3	RR2	Syngenta
26	NS 7709 IPRO	2018	7.4	RR2	Syngenta
27	95R51 RR	2013	5.3	RR	Dupont Pionner
28	TMG 7062 IPRO	2016	6.5	RR2	Tropical Melhoramento e Genética

RR roundup ready®, RR2 roundup ready 2. Source: Elaborated by the authors.

sea level [asl]) and low altitude (336–480 m asl) (Table 2). According to the Köppen climate classification (Alvares et al. 2013), high and low altitudes are described as Cfb and Cfa climates, respectively.

The experimental design was a randomized complete block with three replicates. The experimental plots consisted of 4 × 5 m rows spaced 0.5 m apart, totaling 10 m^2, with a seed density of 34 plants m^2. Agricultural practices were performed in accordance with the technical recommendations for soybean crops.

Table 2. Environment (Env), test locations, edaphoclimatic regions (ECR), geographical coordinates (latitude, longitude, and altitude), and crop season of soybean cultivars.

Env	Test location	Crop season		ECR	Lat	Long	Alt (m als)	Clima
		2017/18	2018/19					
High altitude	Abelardo Luz – SC	x	x	102	26.53S	52.29W	846	Cfb
	Campos Novos – SC	x		103	27.40S	51.23W	963	Cfb
	Guarapuava – PR	x	x	103	25.46S	51.67W	950	Cfb
Low altitude	Medianeira – PR	x	x	201	25.26S	54.08W	414	Cfa
	Palotina – PR	x		201	24.34S	53.83W	336	Cfa
	Realeza – PR	x	x	102	25.77S	53.53W	480	Cfa

Cfa: Humid subtropical, oceanic climate, without dry season, with hot summer; Cfb: Humid subtropical, oceanic climate, without dry season, with temperate summer, according to Köppen's classification (Alvares et al. 2013). Source: Elaborated by the authors using data from Alvares et al. (2013).

Harvest, dry and sample standardization

In the physiological maturity stage (R8) (Fehr and Caviness 1977), the two central lines of each plot were harvested, totaling a useful area of 5 m^2. For storage, the seed samples were dried at 35 °C, until moisture standardization was 13%. The samples were stored in a shed (without temperature and humidity control) for 180 days. We use shed conditions, as this is the real condition of Brazilian farms.

Evaluated traits

Seed physiological tests were performed at the Seeds Laboratory of the Federal University of Technology – Paraná, Campus Pato Branco, PR, Brazil. Germination tests (GER) were performed according to published guidelines for seed testing (Brasil 2009). Four 50-seed subsamples of each cultivar were distributed among two sheets of germitest paper previously moistened with distilled water at 2.5 × the weight of the dry paper and maintained at 25 °C with a photoperiod of 12 h in a germinator. The percentage of normal seedlings was evaluated eight days after the test was established.

Seeds were subject to an accelerated aging test (AA), where 200 seeds were used and divided into four 50-seed subsamples. Seeds were placed on a metallic stainless mesh inside plastic "gerbox" boxes containing 40 mL of distilled water. The boxes were maintained at a temperature of 41 °C for 48 h in a germination biochemical oxygen demand (BOD) chamber. After this period, the seeds were subjected to GER; germinated seeds were counted on the fifth day, and the results are expressed as percentages (Kryzanowski, 1999).

Emergence speed index (ESI) testing was performed under field conditions using four subsamples of 25 seeds. The number of germinated seeds was counted every day until the eighth day after sowing. Seeds were considered germinated when they exhibited cotyledons above the soil surface. The ESI was calculated according to the formula proposed by Maguire (1962), as follows (Eq. 1):

$$ESI = N1/D1 + N2/D2 + Nn/Dn \quad (1)$$

where N1 = number of seedlings emerged on the first day, Nn = accumulated number of emerged seedlings, D1 = first count day, Dn = number of days counted after sowing.

For the oil content (OIL, g·kg^{-1}), protein content (PROT, g·kg^{-1}), and fatty acids, palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3), were performed three replicates, using a near-infrared reflectance spectroscopy (NIR) model Perten DA 7250, expressed on a dry weight basis. The equipment has all the calibration curves.

Statistical analysis

Data were subjected to a joint analysis of variance in a factorial scheme. Mean grouping was performed using the Scott–Knott test ($p < 0.05$). Analysis of variance and means test were performed using "ExpDes.pt" package (Ferreira et al. 2018). Pearson's correlation analysis for high and low altitude environments were performed using *corr_plot()* of "metan" package (Olivoto and Lúcio 2020).

Mean and stability analyses were performed using the *gge()* function of the "metan" package (Olivoto and Lúcio 2020). The parameters used in graphical analysis were data transformation (transform = 0, without transformation), data scale (scaling = 1, data scaled according to the standard deviation), data centering (centering = 2, focused on genotype + genotype × environment effects [G+GE]), and singular value partition (SVP = 1, focusing on the genotype). All statistical analyses were performed using R software (R Development Core Team 2019).

RESULTS

Analysis of variance revealed a significant effect of cultivar × environment interaction (σ^2_{ge}) for all traits evaluated (Table 3). Furthermore, the cultivar effect was significant for most traits, except for palmitic acid (18:0). Environmental effects had a significant impact on GER, AA, OIL, PROT, aspartic acid (ASP), and glutamic acid (GLU). The coefficient of variation (CV) ranged from 2.14% to 32.36%. Heritability values were high, except for ESI and 18:0.

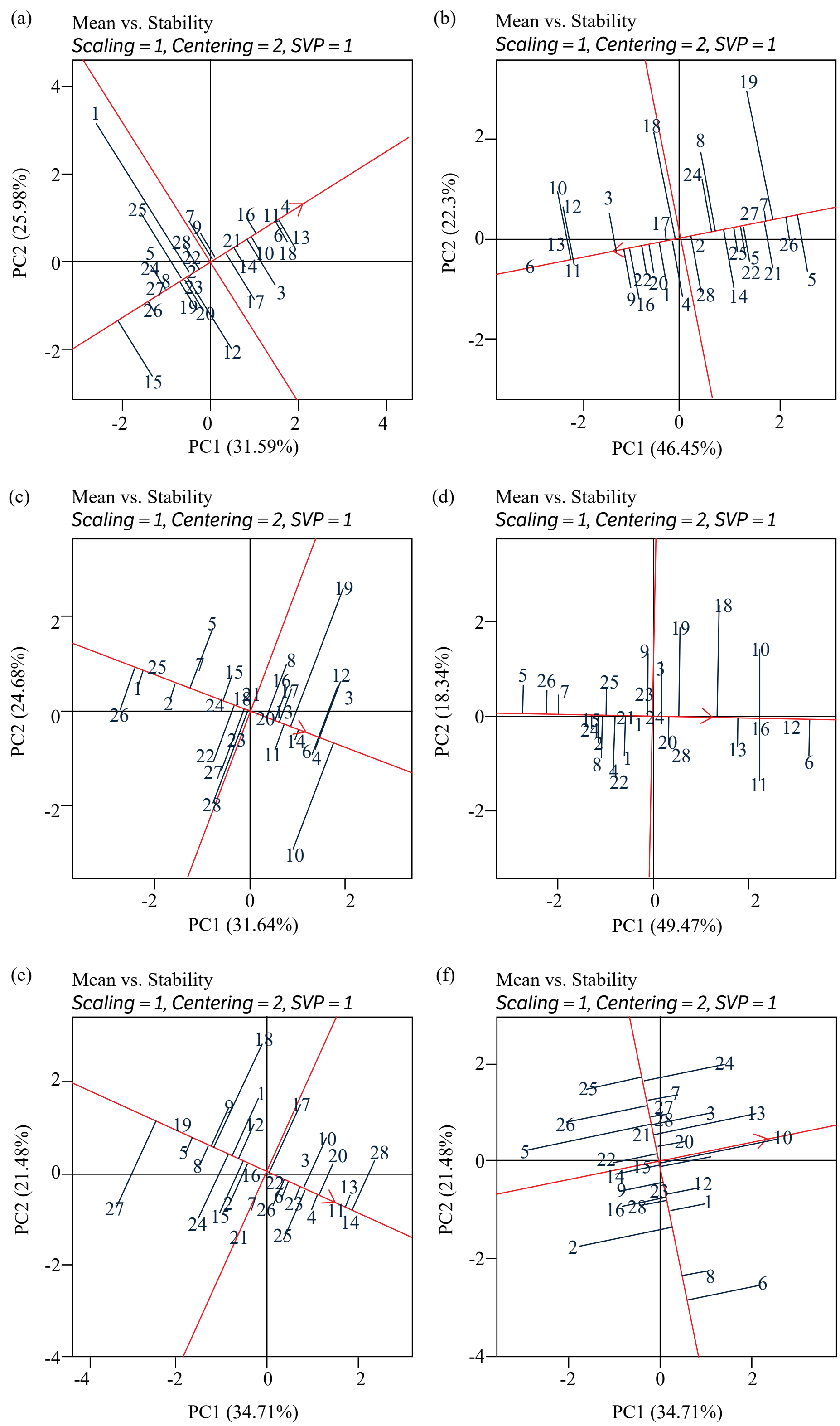
In both environments (high and low altitude), cultivars with good performance were identified. The cultivars 58I60RSF IPRO, M5705 IPRO, M5730 IPRO, and M5838 IPRO showed excellent performance for GER, AA, and ESI at high and low altitudes (Fig. 1). In relation to OIL, cultivars with mean OIL performance were identified at high altitude (NA 5909 RG; 7166RSF IPRO and 95R51 RR) and low altitude (50I52RSF IPRO; 7166RSF IPRO; and NA 5909 RG). Among these cultivars, we highlighted the cultivars NA 5909 RG, 7166RSF IPRO, 95R51 RR and 50I52RSF IPRO, because of their excellent performance in both altitude environments (Fig. 2a and b).

For PROT, the superior cultivars at high altitudes were AS 3730 IPRO, NS 7709 IPRO and, 5855RSF IPRO, and at those at low altitudes were 5855RSF IPRO, AS 3730 IPRO and NS 6906 IPRO. In both altitude environments, the cultivars AS 3730 IPRO and 5855RSF IPRO displayed good performance (Fig. 2c and d).

Table 3. Genetic parameters and germination (GER, %) means, accelerated aging (AA, %), emergence speed index (ESI), oil content (OIL, %), protein content (PROT, %), palmitic acid (16:0, %), stearic acid (18:0, %), oleic acid (18:1, %), linoleic acid (18:2, %), linolenic acid (18:3, %), aspartic acid (ASP, %), glutamic acid (GLU, %).

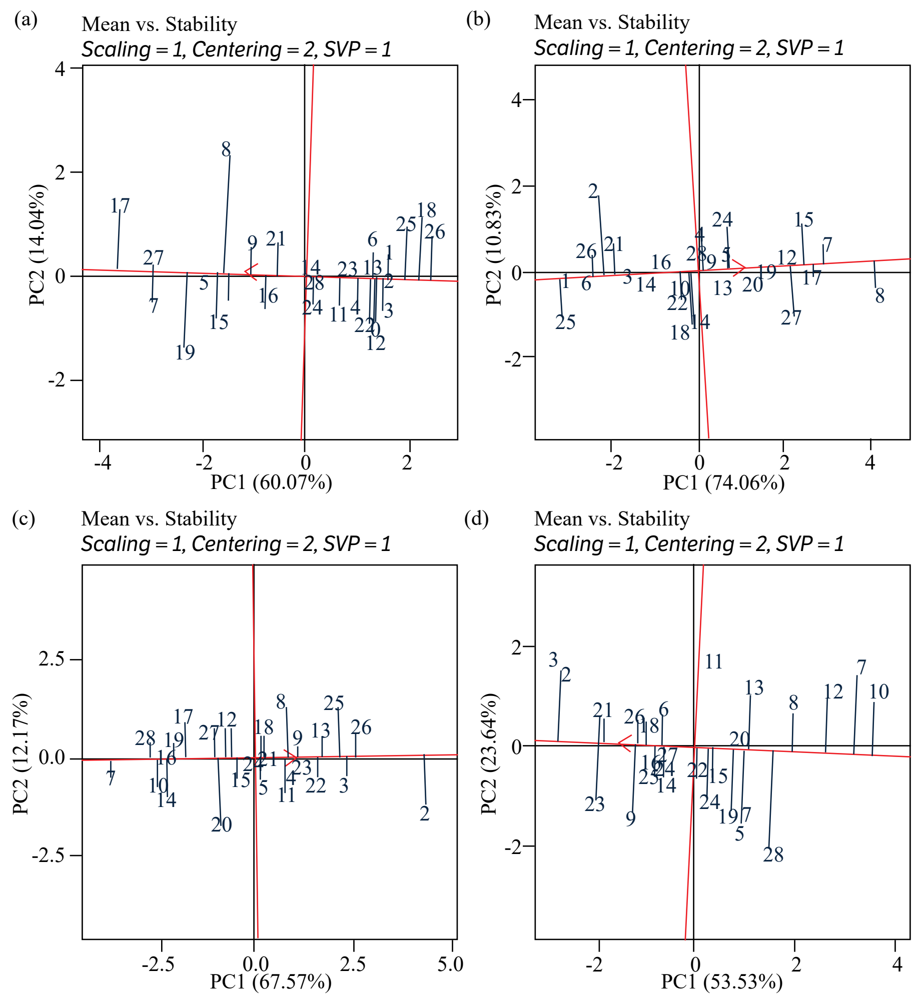
Parameter	GER	AA	ESI	OIL	PROT	16:0	18:0	18:1	18:2	18:3	ASP	GLU
h^2	0.71	0.74	0.26	0.86	0.76	0.72	0.36	0.79	0.65	0.76	0.80	0.73
σ^2_g	74.76**	65.70**	0.21^{ns}	0.38**	0.61**	0.03**	0.00^{ns}	1.98**	1.47**	0.22**	0.01**	0.02**
σ^2_{ge}	48.70**	34.08**	1.01**	0.11**	0.34**	0.02**	0.01**	0.86**	1.18**	0.11**	0.00**	0.01**
σ^2_e	132.7**	300.8**	0.21^{ns}	1.74**	1.73*	0.02^{ns}	0.00^{ns}	0.00^{ns}	0.00^{ns}	0.22^{ns}	0.02**	0.09**
σ^2_{res}	231.25	221.72	3.83	0.29	0.75	0.12	0.07	2.87	6.02	0.50	0.01	0.03
Mean	59.96	46.02	8.41	21.92	37.42	10.92	3.72	21.57	55.65	7.38	4.45	6.73
CV (%)	25.36	32.36	23.27	2.47	2.32	3.16	6.88	7.85	4.41	9.53	2.14	2.57

**,*p < 0.01and p < 0.05; σ^2_g: cultivar variance;σ^2_{ge}: cultivar × environment interaction variance; σ^2_e: environment variance; σ^2_{res}: residual variance; h^2: heritability; CV: coefficient of variance. Source: Elaborated by the authors.



PC: principal component; SVP: singular value partition. See Table 1 for a full description of soybean cultivars. Source: Elaborated by the authors.

Figure 1. Means and stability of the set of 28 soybean cultivars for percentage of germination (GER) performed at high (a) and low (b) altitudes; for accelerated aging (AA) performed at high (c) and low (d) altitudes; and for emergence speed index (ESI) at high (e) and low (f) altitudes.



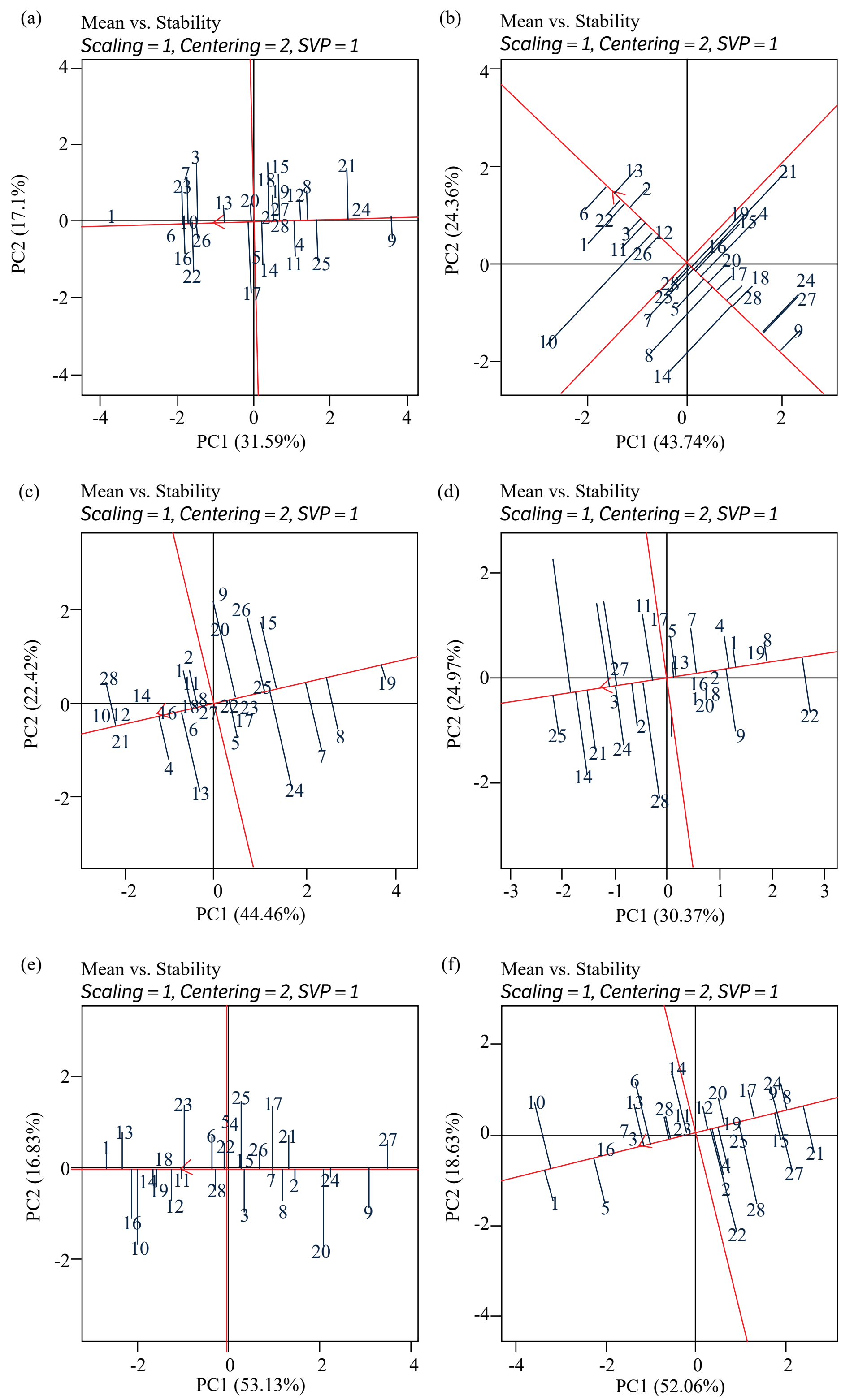
PC: principal component; SVP: singular value partition. See Table 1 for a full description of soybean cultivars. Source: Elaborated by the authors.

Figure 2. Mean and stability of the set of 28 soybean cultivars for oil content (OIL) performed at high (a) and low (b) altitudes; and for protein content (PROT) performed at high (c) and low (d) altitudes.

The mean seed fatty acid profile values of the 28 soybean cultivars evaluated in the high and low altitude environments are shown in (supplementary Table S1). The mean and stability analysis for palmitic acid (16:0) showed better performance for cultivar AS 3610 IPRO at high altitudes (Fig. 3a), and 58I60RSF IPRO at low altitudes (Fig. 3b). Regarding stearic acid (18:0), the highlighted cultivars at high altitudes were M5705 IPRO, TMG 7062 IPRO and NS 6601 IPRO, and at low altitudes were NS 7300 IPRO, M5705 IPRO and M5947 IPRO (Fig. 3c and d). In relation to the fatty acids linoleic (18:2) and linolenic (18:3), lower contents are desirable. Thus, the cultivar with lower content for 18:2 was NS 6909 IPRO, and to 18:3 the NS 95R51 IPRO and 7166RSF IPRO showed greater performance at high altitudes, and NS 6601 IPRO and 58I60RSF IPRO at low altitudes (supplementary Table S1, Fig. 3e and f).

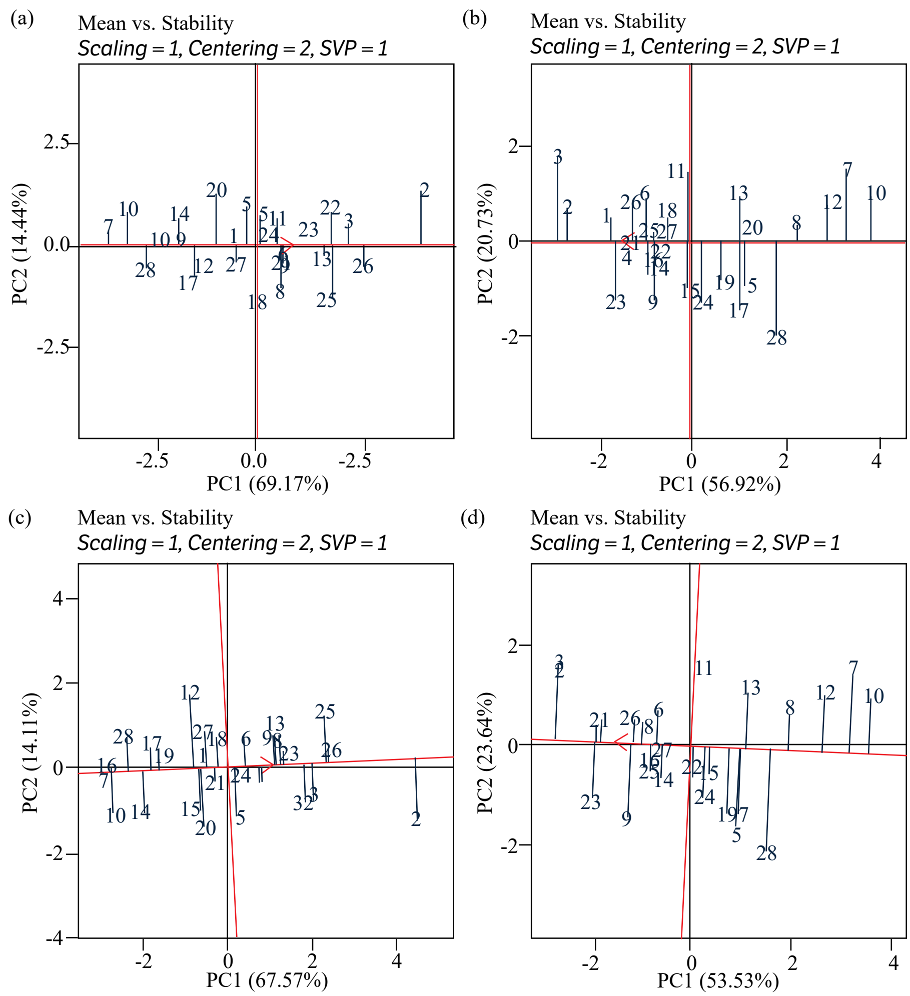
In addition to demonstrating the high environmental stability of these cultivars, it is worth highlighting that their fatty acid composition is closer to the ideal for human consumption and biodiesel production. The cultivars AS 3730 IPRO and 5855RSF IPRO stood out for amino acid content, ASP, and GLU, in both environments (Fig. 4).

Regarding Pearson's correlation coefficient, a positive association between GER × AA ($r = 0.9^{***}$) was observed; thus, these traits are important for describing the physiological quality of seeds (Fig. 5). Furthermore, GER and AA showed a positive association with ESI ($r = 0.61^{***}$ and $r = 0.58^{***}$, respectively) and PROT ($r = 0.40^{**}$, $r = 0.49^{***}$). Physiological traits



PC: principal component; SVP: singular value partition. See Table 1 for a full description of soybean cultivars. Source: Elaborated by the authors.

Figure 3. Mean and stability of the set of 28 soybean cultivars for palmitic acid (16:0) performed at high (a) and low (b) altitudes; and for stearic acid (18:0) performed at high (c) and low (d) altitudes; for linolenic acid (18:3) at high (e) and low (f) altitudes.



PC: principal component; SVP: singular value partition. See Table 1 for a full description of soybean cultivars. Source: Elaborated by the authors.

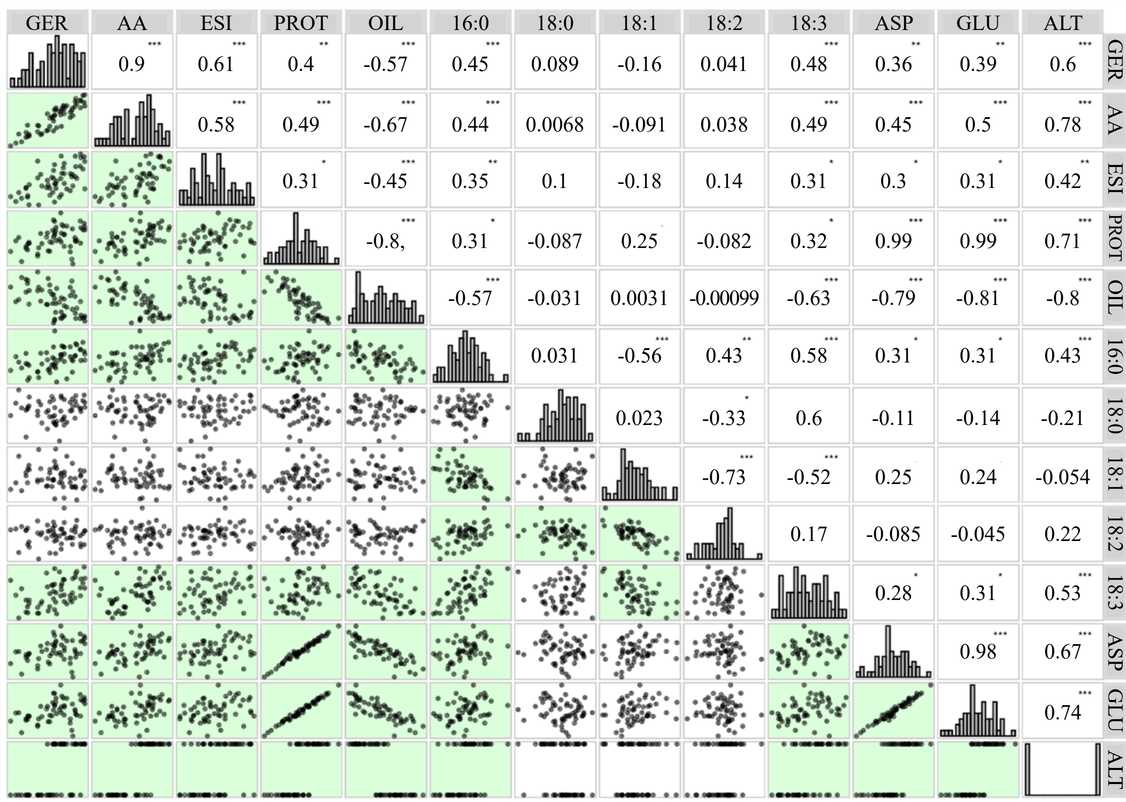
Figure 4. Mean and stability of the set of 28 soybean cultivars for aspartic acid (ASP) performed at high (a) and low (b) altitudes; and for glutamic acid (GLU) performed at high (c) and low (d) altitudes.

(GER, AA, ESI) showed a negative relationship with OIL, showing high and moderate negative association involving GER × OIL ($r = -0.57^{***}$), AA × OIL ($r = -0.67^{***}$), and ESI × OIL ($r = -0.45^{***}$).

Altitude environment (ALT) was positively associated with AA, GER, and ESI (Fig. 5). Therefore, seed production in high-altitude regions can improve physiological quality. Altitude positively affected protein content, with a strong association ($r = 0.71^{***}$), and negatively affected oil content (ALT x OIL, r=-0.80***). A negative relationship was identified between OIL × PROT ($r = -0.83^{***}$), and positive associations were identified involving ALT and ASP and ALT × GLU.

Fatty acids that were positively associated with ALT were 16:0 and 18:3 (Fig. 5). The 16:0 acid showed a positive relationship with GER, AA and ESI, highlighting the effects of monounsaturated fatty acids on the physiological potential of seeds. It is worth to mention, the positive correlation between physiological traits and ASP (GER: $r = 0.36^{**}$, AA: $r = 0.45^{***}$, ESI: r: 0.3^{*}) and GLU (GER: $r = 0.39^{**}$, AA: $r = 0.50^{***}$, ESI: $r = 0.31^{*}$) (Fig. 5).

The correlation reported among the fatty acids reveals their synthesis routes. A highly negative correlation was observed between 18:1 × 18:2 ($r = -0.73^{***}$) and 18:1 × 18:3 ($r = -0.52^{***}$) (Fig. 5), indicating that one acid is derived from the other through the desaturation process enzymatically mediated by FAD_2. It is worth highlighting the positive correlation between ASP × GLU ($r = 0.98^{***}$), and among the amino acids and PROT (ASP × PROT: $r = 0.99^{***}$; GLU × PROT: $r = 0.99^{***}$), confirming that these amino acids are related to protein content.



GER: germination; AA: accelerated aging; ESI: emergence speed index; PROT: protein content; OIL: oil content; 16:0: palmitic acid; 18:0: stearic acid; 18:1: oleic acid; 18:2: linoleic acid; 18:3: linolenic acid; ASP: aspartic acid; GLU: glutamic acid; ALT: altitude. ***,**,* Statistically significant at 0.1%, 1% and 5% probability of error according to Student's *t*-test. Correlations highlighted in green were significant (n = 224). Source: Elaborated by the authors.

Figure 5. Pearson's correlation coefficients involving altitude environment, physiological traits and seed chemical composition of soybean cultivars.

DISCUSSION

In the present study, we observed that seeds produced in the high-altitude environment, where the mean temperature was mild, had higher GER and AA than seeds produced at low altitude (supplementary Table S2).

Thermal stress during the filling stage of soybean seeds can reduce germination and vigor (Nakagawa et al. 2020). High temperatures (~36 °C) can reduce soybean seed germination by 50% (Chebrolu et al. 2016). This reduction in physiological potential may be related to changes in the composition of cellular membranes, especially the fatty acids of the phospholipid bilayer, which results in ion leakage and even loss of structural function (Taiz et al. 2017).

Temperature also affects the oil, protein content, and fatty acid profile of soybean seeds (Alsajri et al. 2020; Bellaloui et al. 2017b; Mourtzinis et al. 2017). In the present study, the difference between the high and low altitudes for oil content was 1.9% (supplementary Table S2). The higher oil content in the low altitude (22.9%) vs. high altitude (21.0%) can be explained by higher temperatures during the grain-filling stage. For protein in the low altitude (36.4%) vs. high altitude (38.4%) can be explained by milder average temperatures. According to Nakagawa et al. (2018), increases in oil content are due to the expression of genes such as Gm DREBL and GmWRI1-like1. These authors reported that high temperatures can reduce protein content, resulting in lower expression of Gm Gy1, Gm Gy2, Gm Gy4, Gm Gy5, and Gm β-conglycinin, which are responsible for protein synthesis. This explains the negative association between oil and protein content (Fig. 5).

Regarding saturated fatty acids (palmitic [16:0], stearic [18:0], and oleic [18:1], small variations were observed between the high and low altitude environments. Therefore, there was variability among the cultivars. Linolenic acid (18:3) presented a greater range between the high and low altitudes, with 7.7% and 7.0%, respectively (supplementary Table S1). Polyunsaturated fatty acids (18:2 and 18:3) are responsible for decreasing the oxidative stability of soybean oil, which reduces the quality and durability of biofuels subsequently produced (Konda et al. 2020).

Altitude was positively associated with protein content ($r = 0.71^{***}$) and negatively associated with oil ($r = –0.80^{***}$). Seed protein content is a complex trait and is highly affected by genotype × environment interactions. Patil et al. (2017) emphasized that, despite protein content being related to cultivar cycle, the geographic region is responsible for part of the observed variation, even more so than the maturity group.

A negative association was identified between OIL × PROT ($r = –0.83^{***}$) (Fig. 5). Elevated temperatures potentiate increased levels of oil and reduce protein accumulation (Alsajri et al. 2020; Chebrolu et al. 2016). Higher altitude environments tend to have higher PROT than OIL (supplementary Table S2). Proteins are important for dicotyledonous species, being mobilized in greater quantities to the embryo during the germination stage and helping to establish seedlings in the field (Han and Yang 2015). Bellaloui et al. (2017b) observed germination rates > 80% in soybean genotypes with higher protein content. Chebrolu et al. (2016) evaluated the effect of heat stress on seed development and reported lower protein concentrations at high temperatures (42 °C), resulting in decreased germination rates.

Protein content showed a positive relationship with physiological potential (PROT × GER: $r = 0.40^{**}$, PROT × AA: $r = 0.49^{***}$). Bellaloui et al. (2017a) reported a positive correlation between germination and accelerated aging with PROT ($r = 0.5^{***}$, $r = 0.3^{***}$), and a negative correlation with OIL ($r = –0.5^{***}$, $r = –0.3^{***}$), similar to the findings of this study. A positive association between fatty acid 16:0 and physiological traits corroborated the results of Bellaloui et al. (2017b).

The negative association between OIL and GER ($r = –0.57^{***}$) and AA ($r = –0.57^{***}$) can be explained by lipid metabolism in soybean seeds under stress conditions. The phospholipase Dα1 (PLDα1) gene is related to oil biosynthesis and is involved in regulating seed maturation and deterioration under high temperatures and humidity (Fang et al. 2017). Zhang et al. (2019) found that phospholipase Dα1-knock-down (PLDα1KD) gene silencing may reduce seed deterioration and improve seed nutritional quality and vigor, without decreased seed oil contents. The reduced activity of desaturation enzymes reduces the levels of reactive oxygen species (ROS) during seed development and germination, thus improving tolerance to environmental stress and allowing wide adaptation to growing conditions.

Soybean breeding programs aim to increase oleic acid (18:1) to obtain better oil quality. The negative association between fatty acids 18:1 × 18:2 ($r = –0.73^{***}$) and 18:1 × 18:3 ($r= –0.52^{***}$) was observed. For biodiesel production, lower concentrations of monounsaturated fatty acids are desirable, mainly because of the low rate of oxidation and decreased nitrogen oxide emissions (Sierra-Cantor and Guerrero-Fajardo 2017). Thus, the search for higher monounsaturated fatty acid levels indirectly contributes to improving the physiological quality of seeds, due to decreasing fatty acid desaturation and ROS. Bellaloui et al. (2013), reported low sensitivity of palmitic and stearic acids to changes in temperature and water stress, while the fatty acids oleic, linoleic, and linolenic acids changed under stress conditions, corroborating the results obtained in this study (Table 3).

Temperature is the main factor affecting seed chemical composition (Abdelghany et al. 2020). Alsajri et al. (2020) observed a decrease in polyunsaturated acids (18:2 and 18:3) at high temperatures and an increase of 18:1. This can be explained by the enzymic activity of ω-6 desaturase. This enzyme is encoded by the FAD2-1A gene, which is responsible for the accumulation and conversion of oleic acid to linoleic and linolenic acids, which are degraded and inactivated at high temperatures (Bellaloui et al. 2013).

Few studies have investigated the effects of amino acid composition and environmental conditions on GLU and ASP. Positive and strong correlations were observed between PROT × ASP ($r= 0.99^{***}$) and PROT × GLU ($r = 0.99^{***}$), and a negative association between OIL × ASP ($r = –0.79^{***}$) and OIL × GLU ($r = –0.81^{***}$). Wang et al. (2019) highlighted that higher levels of ASP, GLU, and PROT are related to protein synthesis. Furthermore, when carbon skeletons are used in protein production, less is available for lipid synthesis. Patil et al. (2017) reported that glutamic acid is the main protein component (19%), followed by leucine (8%), arginine (8%), lysine (7%), and aspartic acid (7%).

In both altitude environments, the cultivar NS 6909 IPRO (24) exhibited the highest mean of 18:1 (Fig. 4a and b). Breeding programs that select adaptable cultivars to determine traits such as 18:1 can develop cultivars for different locations and maintain high oleic acid content at the same time (Oliva et al. 2006). Abdelghany et al. (2020) emphasized the importance of knowing the genotype × environment interactions involved in fatty acid composition to improve the quality of the chemical and physiological composition of soybean seeds.

In summary, the observed positive correlation involving germination and vigor (AA) with protein content and its components (aspartic and glutamic acid) stands out. The physiological potential of the seed was enhanced in higher altitude environments because of the higher seed protein content generated in these environments, which represents a source of energy for the embryo during the germination process and helps in the establishment of seedlings in the field (Wei et al. 2020). Furthermore, the increase in oil content in low-altitude environments was negatively associated with the physiological potential of the seeds. According to Pal et al. (2016), seed quality contributes 20%–25% to crop productivity. In this context, high-altitude environments should be chosen in order to maximize soybean quality.

The growing demand for food challenges breeding programs in their search for increasingly productive and adapted genotypes. Thus, linking genetics to target populations of environments ensures seed quality, benefiting the entire soybean production chain.

CONCLUSION

High-altitude environments result in seeds with high protein content, and improve physiological quality.

Low-altitude environments produce seeds with high oil content. Thus, to recommend specific environments may maximize soybean quality.

AUTHORS' CONTRIBUTION

Conceptualization: Capelin M. A.; Madella L. A. and Panho M. C.; **Methodology:** Capelin M. A. and Rodrigues A. P. D. C.; **Investigation:** Capelin M. A.; Madella L. A.; Panho M. C. and Barrionuevo F.; **Writing – Original Draft:** Capelin M. A.; Madella L. A.; Panho M. C.; Meira D. and Barrionuevo F.; **Writing – Review and Editing:** Capelin M. A.; Madella L. A.; Panho M. C. and Meira D.; **Supervision:** Rodrigues A. P. D. C. and Benin G.

DATA AVAILABILITY STATEMENT

The data will be available upon request.

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APPENDICES

Supplementary Table 1. Fatty acids contents—palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3)—and amino acids—aspartic (ASP) and glutamic (GLU)—of 28 soybean cultivars propagated in high and low altitude environments.

Cultivars	16:0		18:0		18:1		18:2		18:3		ASP		GLU	
	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
AS 3610 IPRO	11.7 Aa[1]	11.1 Ba	3.8 Aa	3.7 Aa	18.2 Be	20.2 Ad	57.4 Ac	55.0 Ba	8.7 Aa	8.4 Aa	4.5 Ad	4.5Aa	6.9 Ad	6.8 Aa
AS 3730 IPRO	11.1 Ac	11.1 Aa	3.7 Aa	3.8 Aa	22.0 Ac	22.9 Ab	56.7 Ac	55.7 Aa	7.2 Ac	6.8 Ac	4.8 Aa	4.5Ba	7.4 Aa	6.8 Ba
5855RSF IPRO	11.2 Ab	11.1 Aa	3.8 Aa	3.9 Aa	22.6 Ac	21.4 Ac	55.5 Ad	54.0 Ab	7.7 Ab	7.6 Ab	4.7 Ab	4.5Ba	7.2 Ab	6.8 Ba
63I64RSF IPRO	10.9 Ac	10.8 Ab	3.8 Aa	3.6 Bb	20.1 Ad	21.1 Ac	56.4 Ac	56.1 Aa	7.7 Ab	6.9 Bc	4.6 Ac	4.4Ba	7.0 Ac	6.6 Bb
68I70RSF IPRO	11.0 Ac	10.7 Bb	3.6 Ab	3.7 Ab	21.1 Ad	19.7 Bd	57.0 Ac	56.6 Aa	7.8 Ab	8.1 Aa	4.5 Ac	4.3Bc	6.9 Ac	6.4 Bc
58I60RSF IPRO	11.4 Ab	11.2 Aa	3.9 Aa	3.7 Aa	19.8 Bd	21.7 Ac	55.9 Ac	53.9 Bb	7.9 Ab	7.2 Bb	4.6 Ac	4.4Bb	7.0 Ac	6.6 Bb
7166RSF IPRO	11.2 Ab	10.7 Bb	3.5 Bb	3.8 Aa	18.3 Ae	19.5 Ad	60.7 Aa	57.2 Ba	7.3 Ac	7.6 Ab	4.3 Ae	4.1Bd	6.6 Ae	6.1 Bd
50I52RSF IPRO	10.8 Ac	10.6 Ab	3.5 Bb	3.7 Aa	23.4 Bc	20.9 Ac	55.4 Bd	58.3 Aa	7.4 Ac	6.3 Bd	4.6 Ac	4.2Bd	7.1 Ac	6.3 Bc
5958RSF IPRO	10.4 Ad	10.4 Ac	3.6 Ab	3.6 Ab	24.9 Ab	22.9 Bb	55.0 Ad	54.7 Ab	6.8 Ad	6.3 Ad	4.6 Ac	4.4Bb	7.1 Ac	6.6 Bb
M5705 IPRO	11.3 Ab	10.9 Ba	3.9 Aa	3.8 Aa	19.9 Ad	20.0 Ad	57.2 Ac	55.1 Ba	8.4 Aa	8.2 Aa	4.4 Ae	4.1Bd	6.6 Ae	6.0 Bd
M5730 IPRO	10.9 Ac	11.0 Aa	3.7 Aa	3.7 Aa	21.9 Ac	21.4 Ac	53.4 Ae	55.1 Aa	8.1 Ab	7.0 Bc	4.6 Ac	4.3Bb	7.0 Ac	6.5 Bb
M5838 IPRO	10.9 Ac	10.9 Aa	3.9 Aa	3.7 Aa	22.4 Ac	20.0 Bd	56.0 Ac	56.5 Aa	8.1 Ab	6.9 Bc	4.5 Ad	4.1Bd	6.8 Ad	6.2 Bd
M5917 IPRO	11.1 Ab	11.3 Aa	3.9 Aa	3.8 Aa	22.5 Ac	20.7 Bc	53.8 Ae	55.1 Aa	8.8 Aa	7.3 Bb	4.7 Ab	4.3Bc	7.1 Ac	6.4 Bc
M5947 IPRO	11.0 Ac	10.5 Bc	3.8 Aa	3.9 Aa	20.1 Bd	22.8 Ab	56.2 Ac	53.3 Bb	8.2 Aa	7.0 Bc	4.4 Ae	4.4Ab	6.7 Ae	6.6 Ab
M6210 IPRO	10.9 Ac	10.8 Ab	3.5 Bb	3.7 Aa	20.2 Bd	22.3 Ab	57.5 Ac	56.5 Aa	7.7 Ab	6.4 Bd	4.6 Ac	4.4Bb	6.8 Ad	6.5 Bb
M6410 IPRO	11.3 Ab	10.8 Bb	3.8 Aa	3.9 Aa	18.0 Be	20.1 Ad	58.4 Ab	55.5 Ba	8.5 Aa	7.8 Ba	4.4 Ae	4.4Ab	6.6 Ae	6.6 Ab
NA 5909 RG	11.0 Ac	10.7 Bb	3.6 Ab	3.7 Aa	21.4 Ad	21.3 Ac	56.8 Ac	56.3 Aa	7.1 Ac	6.4 Bd	4.5 Ad	4.3Bc	6.8 Ae	6.4 Bc
NS 5445 IPRO	11.1 Ac	10.6 Bb	3.8 Aa	3.8 Aa	20.9 Bd	22.8 Ab	56.4 Ac	52.2 Bc	8.2 Ab	7.4 Bb	4.5 Ac	4.4Bb	6.9 Ad	6.6 Bb
NS 5959 IPRO	10.9 Ac	10.8 Ab	3.3 Bb	3.7 Aa	20.7 Ad	20.7 Ac	55.4 Ad	56.9 Aa	8.5 Aa	6.7 Bc	4.4 Ae	4.3Bc	6.8 Ae	6.5 Bb
NS 6006 IPRO	11.1 Ac	10.7 Bb	3.6 Ab	3.6 Ab	21.9 Ac	22.2 Ab	58.0 Ab	56.4 Aa	6.9 Ad	6.7 Ac	4.5 Ad	4.3Bc	6.9 Ad	6.4 Bc
NS 6601 IPRO	10.6 Ad	10.9 Aa	3.9 Aa	4.0 Aa	23.7 Ab	24.9 Aa	53.9 Ae	52.3 Ac	7.4 Ac	6.1 Bd	4.6 Ac	4.4Ba	6.9 Ad	6.7 Bb
NS 6828 IPRO	11.3 Ab	11.1 Aa	3.6 Ab	3.4 Bc	20.7 Ad	21.8 Ac	56.8 Ac	56.5 Aa	7.9 Ab	7.0 Bc	4.7 Ab	4.4Bb	7.1 Ac	6.5 Bb
NS 6906 IPRO	11.3 Ab	10.8 Bb	3.6 Bb	3.9 Aa	20.1 Ad	21.2 Ac	55.9 Ac	56.3 Aa	8.1 Ab	7.1 Bc	4.6 Ac	4.5Ba	7.1 Ac	6.7 Ba
NS 6909 IPRO	10.7 Ad	10.5 Ac	3.5 Bb	3.8 Aa	26.0 Aa	25.9 Aa	52.1 Ae	51.1 Ac	6.9 Ad	6.3 Bd	4.6 Ac	4.3Bb	7.0 Ac	6.5 Bb
NS 7300 IPRO	10.8 Ad	10.8 Ab	3.5 Bb	3.8 Aa	24.1 Ab	23.4 Ab	54.5 Ad	54.3 Ab	7.9 Ab	6.8 Bc	4.7 Ab	4.4Bb	7.2 Ab	6.6 Bb
NS 7709 IPRO	11.2 Ab	11.0 Aa	3.6 Bb	3.8 Aa	21.1 Ad	20.7 Ac	58.0 Ab	56.8 Aa	7.5 Ac	7.3 Ab	4.7 Ab	4.4Ba	7.2 Ab	6.7 Bb
95R51 RR	10.9 Ac	10.4 Bc	3.7 Ab	3.8 Aa	23.1 Ac	22.9 Ab	55.7 Ac	53.8 Ab	6.5 Ad	6.3 Ad	4.5 Ad	4.4Bb	6.9 Ad	6.6 Bb
TMG 7062 IPRO	11.0 Ac	10.6 Bb	3.9 Aa	3.7 Bb	22.2 Ac	21.1 Ac	53.0 Ae	56.0 Ba	7.8 Ab	6.6 Bc	4.4 Ae	4.2Bc	6.7 Ae	6.3 Bc
Mean	11.0	10.8	3.7	3.8	21.5	21.7	56.0	55.3	7.7	7.0	4.5	4.3	6.9	6.5

[1]Means followed by the same lowercase letter in the column and uppercase in the row do not differ statistically from each other by the Skott–Knott test at 5% probability of error. Source: Elaborated by the authors.

Supplementary Table 2. Percentage germination (GER, %), accelerated aging (AA, %), emergence speed index (ESI), oil (OIL) and protein content (PROT) of 28 soybean cultivars grown in high and low altitude environments.

Cultivars	GER		AA		ESI		OIL		PROT	
	High	Low	High	Low	High	Low	High	Low	High	Low
AS 3610 IPRO	60 [1]Ab	54 Ab	49 Ab	30 Bb	8.10 Ac	7.75 Ac	20.3 Bd	21.6 Ae	38.1 Ad	37.6 Aa
AS 3730 IPRO	64 Ab	49 Bc	50 Ab	25 Bc	8.61 Ac	9.01 Ab	20.4 Bd	21.9 Ae	40.7 Aa	38.1 Ba
5855RSF IPRO	79 Aa	65 Bb	68 Aa	35 Bb	9.79 Ab	7.07 Bd	20.4 Bd	22.2 Ae	39.7 Ab	38.1 Ba
63I64RSF IPRO	82 Aa	50 Bc	75 Aa	26 Bc	10.25 Aa	7.90 Bc	20.6 Bc	22.9 Ad	38.9 Ac	36.8 Bb
68I70RSF IPRO	58 Ab	27 Bd	46 Ab	11 Bd	7.30 Ad	6.88 Ad	21.7 Bb	23.1 Ac	38.5 Ac	35.8 Bc
58I60RSF IPRO	81 Aa	79 Aa	74 Aa	59 Ba	9.52 Bb	10.97 Aa	20.5 Bd	21.9 Ae	38.6 Ac	36.8 Bb
7166RSF IPRO	67 Ab	35 Bd	51 Ab	16 Bd	9.07 Ab	6.72 Bd	22.1 Ba	24.0 Ab	36.5 Ae	34.5 Bd
50I52RSF IPRO	61 Ab	47 Bc	59 Ab	29 Bb	7.68 Bd	10.29 Aa	21.7 Bb	24.5 Aa	38.9 Ac	35.2 Bd
5958RSF IPRO	69 Ab	61 Ab	61 Aa	34 Bb	7.61 Ad	8.37 Ab	21.4 Bb	23.0 Ac	39.0 Ac	37.2 Bb
M5705 IPRO	75 Aa	73 Aa	70 Aa	49 Ba	9.76 Ab	7.90 Bc	20.4 Bd	22.7 Ad	37.1 Ae	34.2 Bd
M5730 IPRO	79 Aa	71 Aa	67 Aa	53 Ba	10.62 Aa	9.07 Bb	20.7 Bc	22.4 Ad	38.8 Ac	36.4 Bb
M5838 IPRO	67 Ab	72 Aa	60 Aa	56 Aa	8.14 Ac	8.78 Ab	20.6 Bc	23.7 Ab	38.0 Ad	34.8 Bd
M5917 IPRO	82 Aa	71 Aa	66 Aa	47 Ba	10.70 Aa	7.45 Bc	20.5 Bd	23.1 Ac	39.3 Ab	35.8 Bc
M5947 IPRO	75 Aa	41 Bd	67 Aa	24 Bc	10.75 Aa	8.02 Bc	20.9 Bc	22.8 Ad	37.2 Ae	36.8 Ab
M6210 IPRO	54 Ab	39 Bd	51 Ab	22 Bc	8.44 Ac	7.67 Ac	21.6 Bb	23.8 Ab	38.1 Ad	36.2 Bc
M6410 IPRO	76 Aa	58 Bb	64 Aa	47 Ba	8.64 Ac	8.73 Ab	21.3 Bb	22.5 Ad	37.2 Ae	36.9 Ab
NA 5909 RG	75 Aa	55 Bb	60 Aa	31 Bb	8.81 Ac	7.99 Ac	22.3 Ba	23.9 Ab	37.5 Ad	35.9 Bc
NS 5445 IPRO	82 Aa	56 Bb	61 Aa	46 Ba	7.62 Ad	7.02 Ad	20.2 Bd	22.8 Ad	38.5 Ac	37.0 Bb
NS 5959 IPRO	63 Ab	40 Bd	54 Ab	37 Bb	6.83 Ad	6.84 Ad	21.8 Bb	23.5 Ac	37.3 Ae	36.0 Bc
NS 6006 IPRO	67 Ab	56 Ab	57 Ab	36 Bb	10.06 Aa	7.70 Bc	21.5 Bb	23.3 Ac	37.9 Ad	35.8 Bc
NS 6601 IPRO	73 Aa	33 Bd	58 Ab	26 Bc	9.01 Ab	7.27 Bc	21.2 Bb	22.1 Ae	38.7 Ac	37.6 Ba
NS 6828 IPRO	65 Ab	39 Bd	58 Ab	28 Bb	9.11 Ab	7.77 Bc	20.4 Bd	22.7 Ad	39.2 Ac	36.4 Bb
NS 6906 IPRO	66 Ab	59 Ab	61 Aa	30 Bb	9.65 Ab	8.63 Ab	20.7 Bc	22.5 Ad	39.0 Ac	37.6 Ba
NS 6909 IPRO	60 Ab	48 Bc	56 Ab	36 Bb	8.10 Ac	6.09 Bd	20.9 Bc	23.1 Ac	38.4 Ac	36.3 Bc
NS 7300 IPRO	60 Ab	41 Bd	49 Ab	25 Bc	9.94 Ab	6.10 Bd	20.2 Bd	21.7 Ae	39.6 Ab	37.0 Bb
NS 7709 IPRO	59 Ab	31 Bd	42 Ab	14 Bd	9.39 Ab	6.42 Bd	20.0 Bd	21.9 Ae	39.8 Ab	37.1 Bb
95R51 RR	60 Ab	40 Bd	56 Ab	23 Bc	6.36 Ad	6.97 Ad	22.1 Ba	23.7 Ab	37.9 Ad	36.7 Bb
TMG 7062 IPRO	65 Ab	47 Bc	55 Ab	37 Bb	10.77 Aa	8.67 Bb	21.0 Bc	22.8 Ad	37.0 Ae	35.5 Bc
Mean	69	51	59	33	8.95	7.86	21.0	22.9	38.4	36.4

[1]Means followed by the same lowercase letter in the column and uppercase in the row do not differ statistically from each other by the Skott–Knott test at 5% probability of error. Source: Elaborated by the authors.