

**Oil, protein and fatty acid profiles of Brazilian soybean cultivars in multi-environmental trials****Gilvani Matei<sup>1</sup>, Cátia Meneguzzi<sup>2</sup>, Leomar Guilherme Woyann<sup>2</sup>, Matheus Henrique Todeschini<sup>2</sup>, Diego Maciel Trevizan<sup>2</sup>, Josiane Conte<sup>2</sup>, Antonio Henrique Bozi<sup>2</sup>, Giovani Benin<sup>2\*</sup>**<sup>1</sup>Nidera Seeds, CEP: 85770-000, Realeza-PR, Brazil<sup>2</sup>Universidade Tecnológica Federal do Paraná- UTFPR. Câmpus Pato Branco. Via do Conhecimento, Km 1. CEP 85503-390 - Pato Branco – PR, Brazil\*Corresponding author: [benin@utfpr.edu.br](mailto:benin@utfpr.edu.br)**Abstract**

Soybean is an important oil and protein source for human and animal food, as well as being used in the production of biofuel. Brazilian soybean is known for its quality, especially with regard to its high protein content. This work aimed to present a screening of Brazilian soybean cropped at different locations, to assess the composition of seed with regard to the protein, oil and fatty acid contents. We screened 46 cultivars, which represent more than 50% of the soybean cultivars produced in the South-Center region of Brazil in recent years, analyzed under six environments. In order to conduct the analysis, a grain sample was used to determine seed protein, oil and fatty acid contents, which was accomplished using near infrared spectroscopy (NIR). All the traits presented a genotype x environment interaction (GEI) ( $p < 0.01$ ). A mean oil content of 20.35% was identified for all the evaluated cultivars. This was superior to that obtained in other countries. The mean protein content was 40.20%, which was expected for the Brazilian soybean. This higher oil content is doubly favorable in Brazilian soybean: firstly, for its use as cooking oil and biofuel; secondly, for the production of high protein soybean meal, once all the oil is removed. The cultivars which presented the highest oil contents were also stable across the evaluated environments. On the other hand, the genotypes with the highest protein content presented low stability across the environments. Regarding the fatty acid composition, Brazilian soybean stands out for its low linoleic acid content, which gives a high oxidative stability to the resulting oil and biofuel. Trait associations were dependent on the location, thus breeders can select plants for specific traits at different locations. Brazilian cultivars present interesting characteristics for use in human and animal food products, and for biofuel production.

**Keywords:** *Glycine max*, genotype x environment interaction, GGE biplot, trait correlation, saturated and unsaturated fatty acids.**Introduction**

Soybean has a high nutritional and functional value and is the main vegetal source of protein and oil. Soybean attracts attention due to its high oil (20% average) and protein (40% average) content (Song et al., 2013; Medic et al., 2014). The oil and protein content of soybean seeds are receiving attention from soybean breeders, in addition to the yield (Mahmoud et al., 2006; Song et al., 2013).

With regards to nutrition, soybean is remarkable as it can supply all the essential amino acids required for human nutrition (Erdman, 2000). The quality of the soybean food products directly relies on the high amount of protein in the kernels (Stanojevic et al., 2011). Soybean is also used in animal feed through soybean meal. In this sense, the increase in grain protein content is essential for commercialization, since the international market rewards for the protein content in the soybean meal (Mourya et al., 2016).

In recent years, there has been a growing demand for alternative fuel sources. Soybean oil can be utilized as a renewable and sustainable raw material for biodiesel production (Thelen & Ohlrogge, 2002; Clemente & Cahoon, 2009; Day, 2013; Wu et al., 2013). In Brazil, soybean oil has

traditionally been used for biofuel production (Bergmann et al., 2013), contributing 77% of the total amount of feedstock utilized for this purpose (Castanheira et al., 2015). The biodiesel produced is mixed with fossil fuel in regulated percentages according to the ANP (Brazilian Petrol, Natural Gas and Biofuel Agency) resolutions. Thus, high oil content in the soybean grains is important due to both its performance in biofuel production and in food products.

Besides the importance of the total oil content, the composition of the fatty acid profile is what defines the grain's purpose. Soybean oil consists predominantly of five fatty acids: palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3) (Mandarino et al., 2005; Ferrari et al., 2005; Medic et al., 2014). Palmitic and stearic acid are saturated and stable to the oxidation process, but their ingestion can cause heart diseases (Hu et al., 1997; Chowdhury et al., 2014). The oleic, linoleic and linolenic fatty acids are unsaturated, which means they may bring human health benefits. However, they are susceptible to the oxidation process, which negatively affects the stability and flavor of the oil (Crapiste et al. 1999; Santos et al., 2013; Sarkar et al., 2015). One of

the main objectives of soybean oil composition breeding programs is a reduction in the fatty acids 18:2 and 18:3, and an increase in the fatty acids 18:1, 18:0, and 16:0. These modifications increase the soybean oil quality and stability without the need for hydrogenation, making the oil suitable for human consumption (Mounts et al. 1988; Oliva et al. 2006). To produce biofuel, many of the characteristics related to the composition of fatty acid are relevant.

Soybean grain composition is strongly influenced by the genotype x environment interaction (GEI) (Zhe et al., 2010; Hu & Wiatrak, 2012; Medic et al., 2014; Hemingway et al., 2015). Environment variability in Brazil can change the behavior of the soybean crop, which affects the quantity and quality of the harvested grains. Generally, as the average temperature increases the quantity of oil increases, while the protein decreases (Wolf et al., 1982; Gunasekera et al., 2006; Naeve & Huerd, 2008). The fatty acid profile, when tested for different environments, is also subjected to change. Under high temperatures, the grains present a higher quantity of saturated fatty acids, however, in lower temperatures there is an increase in the content of unsaturated fatty acids (Wilcox & Cavins, 1992; Carrera et al., 2011).

The characterization of the composition of Brazilian soybean cultivars is important, as different soybean based products require a specific raw material. Thus, the objective of this study was to evaluate the protein, oil and fatty acid profile of Brazilian soybean cultivars currently cultivated in Brazil in multi-environmental trials.

## Results

### *The effect of the genotype x environment interaction on soybean kernel composition*

The ANOVA indicated that there were significant effects for genotype, location and for genotype x environment interaction. The GEI effects were significant for PROT ( $P < 0.01$ ), oil ( $P < 0.01$ ), and the fatty acids: PALM ( $P < 0.01$ ), STE ( $P < 0.01$ ), LIN ( $P < 0.01$ ), LINL ( $P < 0.01$ ), and OLE ( $P < 0.01$ ). The mean oil content was found to be highest at PAL (21.21%), while the lowest was found at REA (19.68%), however, it did not differ from those found for MAB (19.86%), SJI (20.35%) and SMI (20.37%) (Fig. 2A).

The highest mean PROT content was observed at MAB (42.32%), while the lowest (38.58%) was found at COR, however, it did not differ statistically from PAL and REA (38.87% and 39.25%, respectively) (Fig. 2A).

Among the fatty acids, PALM was found to have the highest mean at SMI (9.22%), while the lowest means were observed at COR (8.22%) and PAL (8.29%). The highest mean for STE was found at PAL (3.93%), while the lowest was found at COR (3.59%). COR, however, did not differ significantly from REA (3.64%) (Fig. 2B).

LIN had the highest percentage of all the fatty acids present in the soybean oil. The contents obtained at COR (51.70%) and REA (51.35%) were significantly different from the content obtained at SMI (49.92%).

Mean LINL was found to be lowest at SMI (2.59%), while the highest values were observed at REA and COR (4.86 and 4.76%, respectively). The highest mean values for OLE were found at SJI (24.72%) and PAL (24.63%), while the lowest mean, 21.44%, was found at COR. The other fatty acids present in the soybean kernel, and not considered in this

work, contributed with percentages varying from between 9.19 and 11.16%, at REA and MAB, respectively (Fig. 2B).

### *Mean and stability of grain yield and composition for genotype through the GGE biplot*

Mean and stability of oil and PROT contents, as well as the fatty acid profile, for the soybean genotypes can be visualized graphically through the GGE biplots. In the biplot graphs, the line with a single arrow head is the AEC abscissa. The AEC abscissa passes through the biplot origin and the marker for the average environment, it points towards the higher mean values. The average environment has average PC1 and PC2 scores over all the environments (Yan, 2001). The lines perpendicular to the AEC that pass through the biplot origin are referred to as the AEC ordinate. These ordinates are depicted as double-arrowed lines. The greater the absolute length of the projection of a cultivar, the less stable it is. Furthermore, the average yield of the genotypes is approximated by the projections of their markers through the AEC abscissa (Kaya et al. 2006).

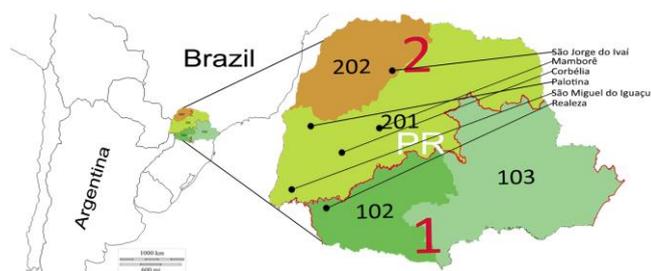
The mean performance and stability of genotype grain yield (Fig. 3) showed that the cultivars NA 5909 RG (2), NS 5959 IPRO (19), M 5917 IPRO (17), M 6410 IPRO (34), NS 6767 (24), NS 6906 IPRO (22), NS 6823 RR (23), NK 7059 RR (25), 5958RSF IPRO (18), BMX Potência RR (26), NS 7000 IPRO (33), NS 7209 IPRO (35), M 6210 IPRO (45), 6563RSF IPRO (31), BMX Turbo RR (13), AS 3610 IPRO (32), 6458RSF IPRO (30), and TMG 7262 RR (27) had the highest yields, while the varieties NA 5909 RG (2), M 5917 IPRO (17), AS 3610 IPRO (32), and 6563RSF IPRO (31) besides having the highest yield also presented high stability. The cultivar BMX AtivaRR (43) presented the lowest yield among the evaluated cultivars.

The mean performance and stability of genotype oil content is presented in Figure 4. The two first principal components (PCs) explained 75.2% of the variation for this characteristic. The cultivars NA 5909 RG (2), 6563RSF IPRO (31), NS 6121RR (38), BMX AtivaRR (43), and SYN1059 RR (12) presented the highest grain oil contents. The lowest oil values were observed for the cultivars NS 6700 IPRO (37), NS 6823 RR (23), NS 6767 (24), NS 7300 IPRO (36), and NS 7209 IPRO (35). The genotypes NA 5909 RG, 6563RSF IPRO, and BMX AtivaRR presented a high oil content and were also stable across the environments. The cultivars NS 6906 IPRO and NS 7209 IPRO were stable, but presented the lowest concentration of oil among the evaluated genotypes.

Mean and stability of genotype grain yield and composition are presented through the GGE biplot, the first two PCs explained 69.3% of the variation for this characteristic (Fig. 5). The cultivars NS 7209 IPRO (35), NS 6700 IPRO (37), NK 7059 RR (25), NS 6906 IPRO (22), BMX Potência RR (26), NS 7300 IPRO (36), DMario 58i (11), NS 5290(7), NS 6823 RR (23), and NS 5258 (6) presented the highest protein contents. The lowest PROT contents were observed for the cultivars NS 6121RR (38), NS 6060 IPRO (39), NA 5909 RG (2), and 6563RSF IPRO (31). Three of these cultivars (6563RSF IPRO (31), NS 6121RR (38), and NA 5909 RG (2)) were among those that presented the highest oil contents. The genotypes with the highest PROT content showed, in general, low stability across the environments, with the exception of the cultivar NS 7300 IPRO (36), which combined both high protein content with a highly stable performance.

**Table 1.** Evaluated cultivars with their respective code, maturity group, year of release and breeding company.

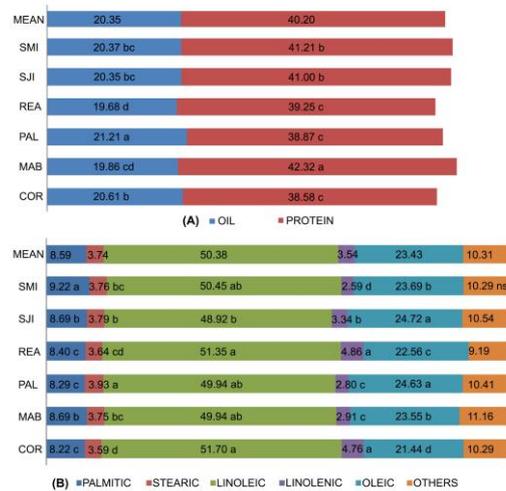
Code	Cultivar	Maturity group	Year of release	Released by
1	NS 5000 IPRO	5	2012	Nidera
2	NA 5909 RG	5.9	2008	Nidera
3	NS 5151 IPRO	5.1	2012	Nidera
4	NS 4823	4.8	2008	Nidera
5	NS 5858	5.8	2010	Nidera
6	NS 5258	5.2	2012	Nidera
7	NS 5290	5.2	2012	Nidera
8	NS 4901	4.9	2012	Nidera
9	BMX Energia RR	5.3	2008	GDM GENÉTICA
10	NS 6262	6.2	2010	Nidera
11	DMario 58i	5.5	2007	GDM GENÉTICA
12	SYN1059 RR	5.9	2010	Syngenta
13	BMX Turbo RR	5.8	2009	GDM GENÉTICA
14	NS 5445 IPRO	5.4	2012	Nidera
15	NS 6006 IPRO	6	2013	Nidera
16	NS 5727 IPRO	5.7	2013	Nidera
17	M 5917 IPRO	5.9	2012	MONSOY
18	5958RSF IPRO	5.8	2012	GDM GENÉTICA
19	NS 5959 IPRO	5.9	2012	Nidera
20	TMG 2158 IPRO	5.8	2013	TMG
21	AS 3570IPRO	5.7	2012	MONSOY
22	NS 6906 IPRO	6.9	2013	Nidera
23	NS 6823 RR	6.8	2013	Nidera
24	NS 6767	6.7	2011	Nidera
25	NK 7059 RR	6.2	2007	Syngenta
26	BMX Potencia RR	6.7	2007	GDM GENÉTICA
27	TMG 7262 RR	6.2	2011	TMG
28	NS 6209	6.2	2012	Nidera
29	NS 6909 IPRO	6.9	2012	Nidera
30	6458RSF IPRO	6	2012	GDM GENÉTICA
31	6563RSF IPRO	6.3	2012	GDM GENÉTICA
32	AS 3610 IPRO	6.1	2012	MONSOY
33	NS 7000 IPRO	7	2012	Nidera
34	M 6410 IPRO	6.4	2011	MONSOY
35	NS 7209 IPRO	7.2	2012	Nidera
36	NS 7300 IPRO	7.3	2012	Nidera
37	NS 6700 IPRO	6.7	2013	Nidera
38	NS 6121RR	6.1	2013	Nidera
39	NS 6060 IPRO	6	2013	Nidera
40	NS 7338 IPRO	7.3	2012	Nidera
41	NS 7237 IPRO	7.2	2012	Nidera
42	NS 5401RR	5.4	2012	Nidera
43	BMX AtivaRR	5.6	2008	GDM GENÉTICA
44	A 6411RG	6.4	2008	Nidera
45	M 6210 IPRO	6.2	2011	MONSOY
46	NS 5106 IPRO	5.1	2012	Nidera



**Fig 1.** Test locations and their respective macro-regions (1 and 2) and microregions (102, 103, 201, and 202) of adaptation of soybean in Brazil. SJ: São Jorge do Ivaí; MAB: Mamborê; PAL: Palotina; COR: Corbélia; SMI: São Miguel do Iguaçu; REA: Realeza.

**Table 2.** Description of the six cultivation sites located in the South region of Brazil.

Location	Code	Latitude	Longitude	Altitude
Corbélia	COR	24°47' S	53°18' W	650 m
Mamborê	MAB	24°19' S	52°31' W	715 m
Palotina	PAL	24°17' S	53°50' W	330 m
Realeza	REA	25°46' S	53°31' W	520 m
São Jorge do Ivaí	SJI	23°25' S	52°17' W	560 m
São Miguel do Guaçu	SMI	25°20' S	54°14' W	290 m

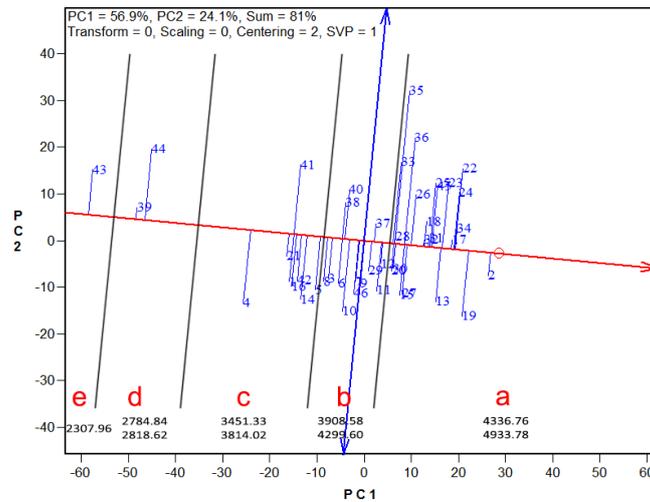


**Fig 2.** Protein, oil and fatty acids of 46 soybean genotypes evaluated in six locations. A) Mean oil and protein content for each location and for all the locations combined. B) Fatty acid content for each location and for the mean of all the locations. SMI, São Miguel do Guaçu; SJI, São Jorge do Ivaí; REA, Realeza; PAL, Palotina; MAB, Mamborê; and COR, Corbélia.

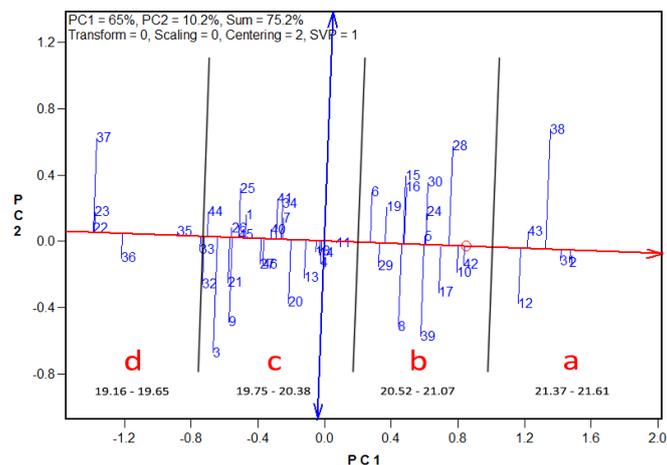
**Table 3.** Pearson coefficient of correlation for grain yield (GY), protein (PROT), oil and the fatty acids palmitic (PALM), linoleic (LIN), linolenic (LINL), oleic (OLE) and stearic (STE).

	COR	MAB	PAL	REA	SJI	SMI	Mean
GY x OIL	-0.17	0.14	-0.19	0.32 *	0.08	-0.29 *	-0.14
GY x PROT	0.43 **	-0.19	0.65 **	-0.33 *	-0.17	0.33 *	0.20
GY x PALM	0.34 *	0.00	-0.04	-0.40 **	-0.18	0.06	0.05
GY x LIN	-0.30 *	0.08	-0.13	0.39 **	0.33 *	-0.07	0.15
GY x LINL	0.21	0.06	-0.03	-0.13	0.01	0.14	0.13
GY x OLE	0.18	0.02	0.26	-0.13	-0.31 *	-0.11	-0.08
GY x STE	0.11	0.19	0.09	-0.26	-0.15	0.13	0.03
OIL x PROT	-0.51 **	-0.60 **	-0.47 **	-0.62 **	-0.34 *	-0.48 **	-0.70 **
OIL x PAL	-0.13	-0.34 *	-0.14	-0.50 **	-0.20	-0.52 **	-0.40 **
OIL x LIN	0.12	0.06	0.01	0.16	0.12	0.33 *	0.00
OIL x LINL	-0.46 **	-0.36 *	-0.38 **	-0.34 *	-0.25	-0.52 **	-0.49 **
OIL x OLE	0.41 **	0.25	0.31 *	0.24	0.18	0.45 **	0.54 **
OIL x STE	0.34 *	0.40 **	0.24	0.22	0.10	0.12	0.29 *
PROT x PALM	-0.03	0.13	-0.11	0.28	-0.12	-0.04	0.19
PROT x LIN	-0.41 **	-0.29 *	-0.20	-0.44 **	-0.22	-0.12	-0.17
PROT x LINL	0.01	-0.08	-0.05	0.08	0.15	-0.02	0.16
PROT x OLE	0.23	0.13	0.16	0.35 *	0.32 *	0.07	-0.07
PROT x STE	0.04	-0.14	0.07	0.13	-0.11	0.09	-0.07
PALM x LIN	-0.29 *	-0.09	-0.13	-0.26	-0.56 **	-0.53 **	-0.47 **
PALM x LINL	0.24	0.33 *	-0.08	0.27	-0.01	0.40 **	0.16
PALM x OLE	-0.37 *	-0.27	-0.15	-0.17	0.03	-0.29	-0.12
PALM x STE	0.15	-0.08	0.26	0.28	0.18	0.02	0.22
LIN x LINL	-0.10	0.23	0.14	-0.24	0.14	-0.19	0.01
LIN x OLE	-0.38 **	-0.74 **	-0.58 **	-0.66 **	-0.58 **	-0.28	-0.60 **
LIN x STE	-0.27	-0.47 **	-0.25	-0.34 *	-0.22	-0.29 *	-0.37 *
LINL x OLE	-0.44 **	-0.63 **	-0.35 *	-0.31 *	-0.53 **	-0.72 **	-0.56 **
LINL x STE	-0.52 **	-0.60 **	-0.65 **	-0.37 *	-0.68 **	-0.64 **	-0.64 **
OLE x STE	0.53 **	0.72 **	0.54 **	0.42 **	0.51 **	0.73 **	0.64 **

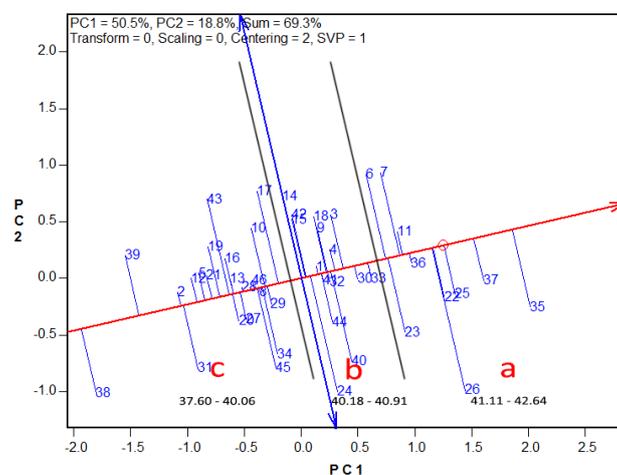
\*\* and \*, significant at 1% and 5% by t test. COR: Corbélia, MAB: Mamborê, PAL: Palotina, REA: Realeza, SJI: São Jorge do Ivaí, SMI: São Miguel do Guaçu.



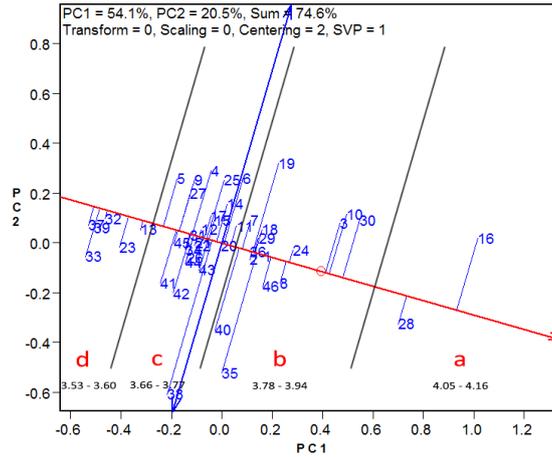
**Fig 3.** GGE biplot for mean productivity and stability, mean clustering via Scott-Knott and the value limits for each cluster of grain yield for 46 Brazilian soybean genotypes cultivated at six locations during the 2014/2015 growing season. PC, principal component; SVP, singular value partitioning method.



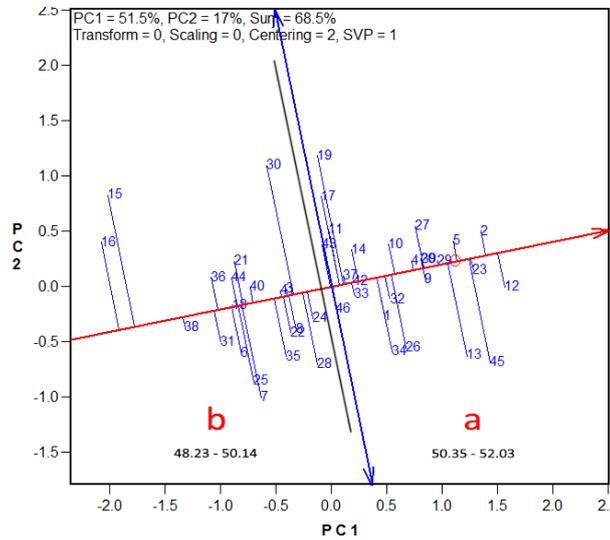
**Fig 4.** GGE biplot for mean and stability, mean clustering via Scott-Knott and the value limits for each cluster for oil content for 46 Brazilian soybean genotypes cultivated at six locations during the 2014/2015 season. PC, principal component; SVP, singular value partitioning method.



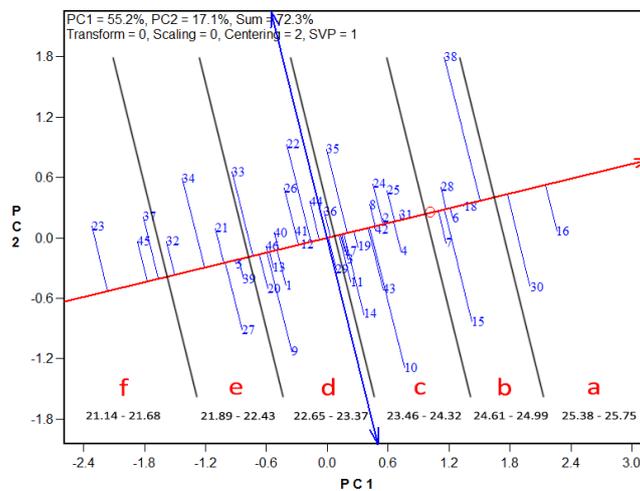
**Fig 5.** GGE biplot for mean performance and stability, mean clustering via Scott-Knott and the value limits for each cluster for protein content for 46 Brazilian soybean genotypes cultivated at six locations during the 2014/2015 season. PC, principal component; SVP, singular value partitioning method.



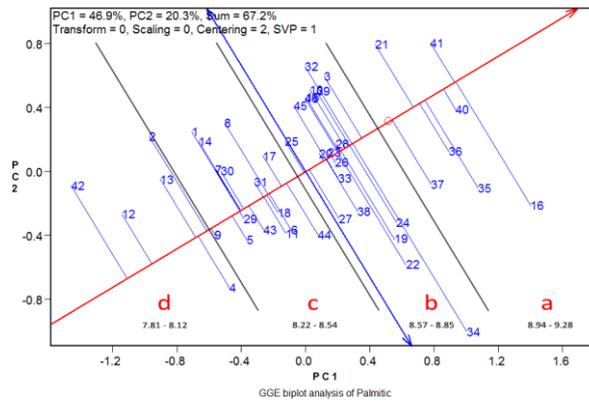
**Fig 6.** GGE biplot for mean performance and stability, mean clustering via Scott-Knott and the value of limits for each cluster for stearic fatty acid content for 46 Brazilian soybean genotypes cultivated at six locations during the 2014/2015 season. PC, principal component; SVP, singular value partition method.



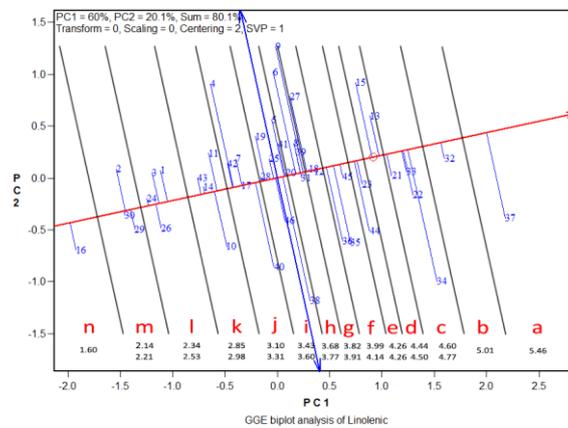
**Fig 7.** GGE biplot for mean performance and stability, mean clustering via Scott-Knott and the value limits for each cluster of linoleic fatty acid content for 46 Brazilian soybean genotypes cultivated at six locations during the 2014/2015 season. PC, principal component; SVP, singular value partition method.



**Fig 8.** GGE biplot for mean performance and stability, mean clustering via Scott-Knott and the value limits for each cluster for oleic fatty acid content for 46 Brazilian soybean genotypes cultivated at six locations during the 2014/2015 season. PC, principal component; SVP, singular value partition method.



**Fig 9.** GGE biplot for mean performance and stability, mean clustering via Scott-Knott and the value limits for each cluster of palmitic fatty acid content for 46 Brazilian soybean genotypes cultivated at six locations during the 2014/2015 season. PC, principal component; SVP, singular value partition method.



**Fig 10.** GGE biplot for mean performance and stability, mean clustering via Scott-Knott and the value limits for each cluster of linolenic fatty acid content for 46 Brazilian soybean genotypes cultivated at six locations during the 2014/2015 season. PC, principal component; SVP, singular value partition method.

Figure 6 allows the visualization of the graphical representation of the mean performance and stability of the genotypes regarding the fatty acid STE. The PCs 1 and 2 explained 74.6% of the total variation. The cultivars NS 5727 IPRO (16), and NS 6209 (28) presented the highest STE fatty acid content. On the other hand, the varieties NS 6700 IPRO (37), NS 6060 IPRO (39), NS 7000 IPRO (33), AS 3610 IPRO (32), NS 6823 RR (23), and BMX Turbo RR (13) had the lowest contents. The genotype NS 6209 (28) was shown to be stable in the evaluated environments. However, the AS 3610 IPRO (32), and BMX Turbo RR (13) genotypes had low contents of this fatty acid, but were shown to be stable in the environments.

The mean performance and stability of the fatty acid LIN can be observed for the genotypes in Figure 7. The two PCs explained 68.5% of the total variation. The cultivars were divided into two groups. The high concentration grouping containing the cultivars SYN1059 RR (12), NA 5909 RG (2), NS 6823 RR (23), M 6210 IPRO (45), and NS 5858 (5). In contrast, the NS 6006 IPRO (15) and NS 5727 IPRO (16) cultivars presented the lowest LIN acid contents. Among the genotypes with the highest LIN acid content, the NS 6823 RR (23) and NS 6909 IPRO (29) genotypes were the most stable. Regarding the genotypes with the lowest LIN acid contents, NS 6121RR (31) and 5958RSF IPRO (18) were the most stable.

For OLE acid, it is possible to note that the two PCs accounted for 72.3% of the total variation (Fig. 8). The NS 5727 IPRO (16) and 6458RSF IPRO (30) cultivars had the highest content of this acid, while the NS 6823 RR (23), M 6210 IPRO (45) and NS 6700 IPRO (37) genotypes had the lowest content. The genotypes with the highest OLE acid contents were not observed as being stable. However, among the genotypes with concentrations superior to the overall mean 5958RSF IPRO (18) and NS 5258 (6) were stable in the evaluated environments. In contrast, among the cultivars with the lowest OLE contents, the genotypes NS 5858 (5) and NS 5106 IPRO (46) were stable.

The biplot graphic for PALM acid is presented in Figure 9. It was observed that the two PCs represented 67.2% of the total variation. The cultivars NS 5727 IPRO (16), NS 7237 IPRO (41), NS 7338 IPRO (40), NS 7209 IPRO (35), NS 7300 IPRO (36), AS 3570IPRO (21), and NS 6700 IPRO (37) presented the highest content of this fatty acid, while the cultivars NS 5401RR (42), SYN1059 RR (12), BMX Turbo RR (13), NS 4823 (23) and NA 5909 RG (2) had the lowest means. Among the genotypes with the highest PALM acid contents, the genotype NS 7338 IPRO (40) was the most stable. For the genotypes with the lowest contents, none were observed to present high stability. The variety BMS Energia RR (9), which was stable, had an inferior PALM acid concentration than the overall mean.

The biplot for the LINL acid content is shown in Figure 10. The two PCs represented 80.1% of the total variation. The highest LINL acid content was obtained for the NS 6700 IPRO (37) cultivar. In contrast, the NS 5727 IPRO (16) cultivar presented the lowest content. These genotypes were not stable. The AS 3610 IPRO (32) and 6458RSF IPRO (30) genotypes were the most stable from the high and low LINL acid content groups, respectively.

### **Trait association**

There were large variations in magnitude, significance, and direction, i.e. positive and negative, for the associations among the traits (Table 3). For GY x PROT, the correlations varied from 0.65\*\* in PAL to -0.33\* in REA. For GY x OIL, the correlation changed from 0.32\* at REA to -0.29 at SMI. In general, the positive association among GY x PROT resulted in a negative association between GY x OIL, under the same environment. STE and LINL acid did not correlate with yield for any of the evaluated environments. LIN acid had a significant correlation with yield at COR (-0.30\*), REA (0.39\*\*), and SJI (0.33\*). The correlation for yield x OLE was significant only for the SJI (-0.31\*) environment.

The correlation between oil x PROT varied between -0.62 (REA) and -0.34\* (SJI). The oil content also presented significant correlations, for the environmental average, with PALM, LINL, and OLE acid, with values of -0.40\*\*, -0.49\*\*, and 0.54\*\*, respectively. For the environmental average, the percentage of protein did not show a significant correlation for any of the fatty acids.

Through the environmental average, some fatty acids had significant correlations among themselves. LIN acid had a significant correlation with PALM, OLE, and STE acid, with values of -0.47\*\*, -0.56\*\*, and -0.37\*, respectively. LINL acid had a significant association with the OLE and STE acid, with values of -0.56\*\* and -0.64\*\*, respectively. The OLE acid was positively correlated with stearic acid for the overall mean of environments (0.64\*\*). For the significant correlations among fatty acids, at some of the environments, the interaction was not significant, however, the direction of the correlation was not altered.

### **Discussion**

#### ***Environmental effect on grain composition***

The ANOVA indicated the presence of a GEI for all the evaluated Traits, showing that the concentrations of protein, oil, and the fatty acids significantly respond to environmental variation. These results are in accordance with other studies (Kumar et al., 2006; Hemingway et al., 2015; Bellaloui et al., 2015; Song et al., 2016).

Besides the genotype, crop management and biotic and abiotic factors such as sowing date, temperature, water conditions, soil conditions, agronomical practices, and disease severity can also affect the composition of the grains (Dardanelli et al., 2006; Bellaloui et al., 2009; Bellaloui et al., 2015; Lee et al., 2015). Generally, the protein content of soybean is increased when the crop is grown under moderate water stress and high temperatures during the grain filling phase (Vollmann et al., 2000; Rotundo & Westgate, 2009; Rotundo et al., 2014). Lower protein contents, on the other hand, may be the result of normal or

above normal water supply and mild temperatures. Besides this, Pípolo (2002) observed that soybean grain protein tended to increase with increasing altitude.

The mean crude PROT content in this work was 40.20%. Grieshop & Fahey Jr (2001) obtained a PROT content of 40.30% for soybean produced in Paraná State – Brazil, in an area similar to those utilized in this study. Karr-Lilienthal et al. (2004) found PROT to be 39.3%, which corroborated the results from our work. Our results indicated that altitude was the observed parameter that differentiated the study locations, with regard to the grain protein content. The highest PROT content was obtained at MAB, which was the environment with the highest altitude (Table 2).

The mean oil content observed was 20.34%. There was a significant difference between the environments, with the lowest values found at REA (19.68%), while the highest values were at PAL (21.21%). The oil contents found for these two sites were higher than those found in other studies. Grieshop & Fahey Jr (2001) obtained oil contents for Brazilian soybean that varied between 18.02% and 19.75% for different Brazilian states. These authors found oil contents to be considerably lower in Chinese soybean. Other studies have associated high temperatures with soybean grain oil quantity (Wolf et al., 1982; Naeve and Huerd, 2008). The environment PAL was found to provide the highest oil content among all the environments. This is explained by the high temperatures during the grain filling phase. On the other hand, the environment REA provided inferior oil content, which was a consequence of the lower temperatures during this phase.

In this study, the average PALM acid contents accounted for 8.58% of the total oil content. These levels were lower than the 12% considered normal for soybean by Wilson et al. (2002) and Fehr (2007). Studies involving Brazilian soybean cultivars demonstrate that it presents lower values than those considered normal for this fatty acid (Santos et al., 2013; Priolli et al., 2015). Nevertheless, specific breeding programs that aim to develop cultivars with differentiated contents of this fatty acid pointed out that genetic modifications have been conducted for the development of genotypes with a lower content (< 4 %) of PALM (Stoltzfus et al., 2000). The normal STE fatty acid content in soybean grains is approximately 4% (Wilson et al., 2002). In this study, the mean content of stearic acid for the environments combined was 3.74%, with values varying from 3.64% (REA) to 3.93% (PAL). Similar results were found by Priolli et al. (2015), who obtained means of 3.2% for Brazilian soybean genotypes.

Unsaturated fatty acids differ in their degree of unsaturation. 18:1 is monounsaturated, while 18:2 and 18:3 are polyunsaturated fatty acids. The mean 18:2 content was 50.38% and it varied from 48.92% (SJI) to 51.7% (COR). The mean values observed in our study were similar to those obtained by both Vieira et al., 1990 and Priolli et al., 2015. We observed that the Brazilian genotypes had lower levels than those considered appropriate ( $\geq 54\%$ ) according to Wilson (2004), but within the range of 48 to 58% indicated by Bellaloui et al. (2015). The content level considered normal for the fatty acid LINL is 8%, according to Wilson (2004). In our study, the mean for this trait was 3.54% and it varied from 2.59% (SMI) to 4.86% (REA). This indicates that the Brazilian soybean genotypes present, on average, a low LINL content (3-6%). These results were lower than those

found by Priolli et al. (2015), who observed a mean content of 6.0%. However, these authors analyzed material obtained from genebanks, which tend to have a greater range of values, and thus increase the average. Soybean cultivars contain roughly 22% OLE fatty acid (Wilson, 2002). In our study, the mean for this trait was of 23.4% and the values varied from 21.44% (COR) to 24.72% (SJI). These data agree with those found by Priolli et al. (2015) for Brazilian soybean genotypes.

The composition of fatty acids can be influenced by temperature (Wolf et al 1982; Cheesbrough, 1989; Tang et al., 2005; Medic et al., 2014). Studies conducted by Ren et al. (2009) and Carrera et al. (2011) showed that high temperatures during seed development significantly increased the total oil content, and the contents of the fatty acids OLE, PALM, and STE, however, the contents of LIN and LINL decreased. In our study, the environment PAL, which has a higher mean temperature, presented the highest contents of oil, STE and OLE.

#### ***Cultivars and their protein, oil and fatty acid quality***

The GGE biplot methodology has been widely used to analyze genotype x environment interactions. This methodology has been used in genotype stability and adaptability analysis, to verify the formation of mega-environments and to identify discriminant and representative environments, among other characteristics of relevance to plant breeders (Yan et al., 2007; Lado et al., 2015; Sharma et al., 2016). In this work, the GGE methodology was used to identify genotypes with high stability and superior agronomical performance for the evaluated traits.

The results show that there was differentiation among evaluated genotypes for all the studied variables. Grain yield is one of the main parameters observed when selecting a cultivar. In this sense, the variety NA 5909 RG (2) has a high productivity concomitantly with a high oil content and phenotypic stability for most of the evaluated variables. The genotypes that had the lowest oil content (NS 6700 IPRO (37), NS 6823 RR (23), NS 6906 IPRO (22), NS 7300 IPRO (36), and NS 7209 IPRO (35)) were those that presented the highest PROT contents. Similarly, the varieties that presented the highest oil contents also had the lowest quantity of protein (NS 6121RR (38), NA 5909 RG (2), 6563RSF IPRO (31), and BMX AtivarR (43)). This explains the negative correlation between these traits, thus, among the studied variables there were no cultivars presenting high contents of both oil and protein. This behavior has also been observed by other authors (Clemente & Cahoon, 2009; Wittkop et al., 2009; Popovic et al., 2012).

One of the main objectives of a soybean oil composition breeding program is the reduction of the fatty acids 18:2 and 18:3 and an increase in 18:1, 18:0, and 16:0 when looking for characteristics related to the oxidative stability of oils (Mounts et al. 1988; Oliva et al. 2006). The genotype NS 5727 IPRO (16) presented high oil oxidative stability potential. This cultivar had a high saturated fatty acid and a low polyunsaturated content, which confers high stability to the oil. However, this variety had an average yield that resulted in it not being among the most productive cultivars. When the soybean is intended mainly for human consumption, a low content of saturated fatty acids is ideal,

because high contents of these fatty acids increase cholesterol, raising the risk of heart disease. The cultivar BMX Turbo RR (13) presented a high grain yield and low saturated fatty acid content. This composition is appropriate when its grains are used for the production of food. Moreover, soybean grains high in 16:0 and 18:0 can be used for high cetane number (CN) biofuel production.

#### ***Correlations are dependent on location***

Correlation analysis can provide gains in plant breeding, as it helps determine the selection strategy and make the selection process less time consuming, further to this, it can reduce the physical space and financial resources used by the breeding program. In our study, the association among traits was strongly influenced by the environment (Tab. 3). For the average correlation, the traits GY x OIL and GY x PROT did not show a significant association. However, the associations varied when analyzed in different environments. REA had a significant positive association between GY x OIL, and a significant negative correlation for GY x PROT. For the SMI environment the correlation among GY x OIL was negative, while GY x PROT was positive. When correlations are constant through the years, breeders can perform plant selections in environments where the desired parameters are simultaneously present. If the breeding program's objective is to find a high protein concentration, the SMI location would be ideal for plant selection, thus, genotypes with high grain yield will also have high protein content. Likewise, if the objective is high oil content, plant selection could be performed at REA, this way genotypes with a high productivity would also possess high oil content, simultaneously.

The GY was not correlated with any of the traits in this study in the joint analysis for the six locations. These results corroborate with Popović et al. (2012) and Bonato et al. (2000) who did not find a significant correlation between GY x PROT and GY x OIL. Many authors have demonstrated a negative correlation between GY x PROT (Thorne & Fehr, 1970; Hartwig & Hinson, 1972; Hymowitz et al., 1972; Voldeng et al., 1997; Wilcox & Guodong, 1997). Others have shown a positive association between PROT and GY (Kwon & Torrie, 1964; Simpson Junior & Wilcox, 1983).

For the associations between OIL x PROT, LIN x OLE, LINL x OLE, LINL x STE, and OLE x STE the correlations were consistent for every environment and for the general mean. There was a significant negative association between oil content and protein. Thus, whenever the oil content is desired in a breeding program, the protein is reduced. The inverse association between soybean oil and protein is well documented (Johnson et al., 1955; Bonato et al., 2000; Clemente & Cahoon, 2009; Wittkop et al., 2009; Popovic et al., 2012). Normally, when the oil content decreases by 1%, the protein content is increased by 2% (Clemente & Cahoon, 2009).

There was a significant negative correlation between oleic fatty acid and the polyunsaturated fatty acids. These data corroborate with other studies (Kumar et al., 2006; Lee et al., 2007; Bachlava et al., 2008; Song et al., 2013). The negative correlation between OLE and LIN can be understood since linoleic acid is formed by the oxidative denaturation of oleic, and therefore, when the LIN content is enhanced OLE acid is reduced (Zhang et al., 2015). STE fatty

acid had a significant negative correlation with LINL acid and a positive correlation with OLE. Zhang et al. (2015) found similar results regarding the correlation between STE and 18:3, however, the correlation with 18:1 was negative. The positive association between oleic and stearic acid can make it difficult to obtain genotypes that present ideal fatty acid composition characteristics.

## Materials and Methods

### Plant materials

We evaluated forty-six Brazilian soybean cultivars that are widely cultivated in the Center-South region of Brazil. The cultivars were released between 2007 and 2013 (Table 1). The cultivars came from the breeding companies Nidera, Monsanto, Dom Mario Group, Syngenta, and TMG, which run the major soybean breeding programs in Brazil. The maturity groups for the Brazilian soybean were inspired by the American method, meaning that the genotypes with lower maturity groups are adapted to locations at higher latitudes.

### Locations

The experiments were conducted at six locations in the state of Paraná – Brazil (Figure 1 and Table 2), all of which classified as climate type Cfa (Köppen-Geiger) (Peel et al., 2007).

### Experimental design

The experiments were conducted in a randomized complete block design, with three replications. The experimental units consisted of four lines of five meters, with a 0.5m row spacing. The sowing rate was 30 seeds m<sup>-2</sup> and there was a base fertilization of 350 kg ha<sup>-1</sup> of NPK (02-20-20). The crop treatments were performed in accordance with their technical recommendations.

### Evaluation of traits related to grain yield and quality composition

The yield, in kg ha<sup>-1</sup>, was obtained by harvesting the two central lines (5 m<sup>2</sup> of useful area). A sample of the harvested kernels was used to determine the protein (PROT), oil, palmitic acid (PALM), stearic acid (STE), oleic acid (OLE), linoleic acid (LIN), and linolenic acid (LINL) contents. These analyses were conducted by near infrared spectroscopy (NIR), using a Foss XDS–NIR Rapid Content Analyzer (FOSS Analytical, Slangerupgade, Denmark). The NIR spectral data were collected between 400 and 2,500 nm at 2 nm intervals. Mathematical procedures on the spectral information were carried out as described by Han et al. (2014).

### Statistical analysis

Analyses of variance (ANOVAs) were performed jointly across locations, with genotype and location being considered as fixed effects. Data normality and variance homogeneity were verified before the analysis. For the environments, we performed a Duncan's test to compare means between the environments. Treatment means were

grouped using a Scott-Knott test through the ScottKnott package (Jelihovschi et al., 2010). Additionally, the Pearson's correlations among the traits were analyzed using the Hmisc package (Harrell & Dupont, 2012). For the Pearson correlation analysis for each location, the experiments were considered randomized blocks. However, to obtain the average correlation for the locations a joint analysis was conducted. The significance of the correlations was verified by *t* test. All procedures were carried out using R software (R Core Team, 2015).

The graphical analysis of mean production and stability was performed using GGEbiplot software (Yan, 2001), which considers the genotype stability associated with its yield means. The following parameters were used: Data transformation (Transform = 0, no transformation), data scale (Scaling = 0, no scale), data centralization (Data centering = 2, genotype plus genotype x environment (G+GE) interaction (GEI)), and singular values partition (SVP = 1, focus on genotype).

## Conclusion

Protein, oil and fatty acids have significant genotype x environment interaction, highlighting the need to evaluate the performance of cultivars at a wide range of locations. Brazilian soybean presented high oil and protein contents. High oil content promotes the use of the soybean in food and biofuel production. Furthermore, high grain oil content helps effectively concentrate the protein content of the meal produced from Brazilian soybean. This makes the Brazilian soybean meal a standard in the international market. These cultivars have different low concentrations of saturated fatty acids, mainly through reduced palmitic acid content, which makes it suitable for human consumption. Furthermore, Brazilian soybean has low levels of linoleic fatty acid, which provides oxidative stability to the oil, and together with a general low content of polyunsaturated fatty acids is an important feature for biofuels.

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