

Analysis of phenotypic plasticity in indeterminate soybean cultivars under different row spacing**Alvadi Antonio Balbinot Junior^{1*}, Maria Cristina Neves de Oliveira¹, Claudemir Zucareli², André Sampaio Ferreira², Flávia Werner², Marcelo Augusto de Aguiar e Silva²**¹Embrapa Soybean, Rodovia Carlos João Strass, distrito de Warta, CEP 86001-970, Londrina, Paraná state, Brazil²Londrina State University, Rodovia Celso Garcia Cid, PR 445, Km 380, Caixa Postal 6001, 86051-980, Londrina, Paraná state, Brazil*Corresponding author: alvadi.balbinot@embrapa.br**Abstract**

This study aimed to evaluate how branch and stem variables contribute to grain yield per plant of two indeterminate soybean cultivars at varied row spacing. Four experiments were conducted in the 2013/14 and 2014/15 cropping seasons under a randomized complete block design with three replications. Each experiment consisted of one row spacing: 0.2 m (narrow row), 0.5 m (traditional), 0.5 m (crossed rows), and 0.2/0.8 m (twin rows). We evaluated two cultivars (BRS 359 RR and BMX RR Potência) and three seeding rates: 150, 300, and 450 thousand seeds ha⁻¹. Regarding phenotypic plasticity, grain production per plant was regarded as a dependent variable, while the number of branches per plant, the percentage of grain production from branches, and yield components of branches and stems as independent variables. Data underwent stepwise regression and principal component analysis. The results showed that number of pods per plant from branches is the most determinant variable of plasticity trait, regardless of row spacing. The number of pods per plant from branches and stems, the number of branches per plant, and the percentage of grain production from branches were associated with the plasticity. The number of grains per pod and the thousand-grain mass from branches and stems had no significant contribution to the soybean plasticity.

Keywords: *Glycine max* (L.) Merrill; row spacing; seeding rate; stem; branches.**Abbreviations:** PROD_grain production per plant; NBPL_number of branches per plant; NPPLB_number of pods per plant from branches; NPPLS_number of pods per plant from stems; NGPB_number of grains per pod from branches; NGPS_number of grains per pod from stems; TGMB_thousand-grain mass from branches; TGMS_thousand-grain mass from stems; PGB_grain production from branches in %; SCWB_sequential climatological water balance; PCA_principal component analysis**Introduction**

In the last decade, there have been significant changes in Brazilian soybean production, such as morphological and physiological changes of cultivars, increasing grain yield expectations, anticipating sowing dates to reduce the incidence of diseases and pests, or even to grow corn in succession. This leads to a research updating towards plant spatial arrangement, aiming to increase grain yield and crop profitability (Balbinot Junior et al., 2015a; Werner et al., 2016). Hence, alternative spatial arrangements such as narrow row spacing (0.20 to 0.30 m) (Balbinot Junior et al., 2015b) and crossed rows have been studied, in which half of the seeds are sown in one direction and the other half to the transverse direction (Balbinot Junior et al., 2015a). Also, twin rows consist of sowing parallel rows alternating spacing, one large and one narrow (Duarte et al., 2016).

The spatial arrangement of soybean plants can be altered by sowing rate, which modifies intraspecific competition for water, light, and nutrients, influencing plant growth, canopy architecture, phytosanitary management, and grain yield (Procópio et al., 2013; Ferreira et al., 2016; Werner et al., 2016). Studies have demonstrated the high phenotypic plasticity of soybean, modifying growth and yield

components as a function of the number of individuals per area. This trait helps maintaining a constant productivity in a wide range of plant densities (Rambo et al., 2004; Lee et al., 2008; Board and Kahlon, 2013; Procópio et al., 2013; De Luca and Hungria, 2014; Suhre et al., 2014; Moreira et al., 2015; Petter et al., 2016).

In Brazil, most of the studies on the phenotypic plasticity of soybean have used cultivars with determinate growth type and vigorous vegetative growth (Rambo et al., 2004). However, the majority of soybean cultivars released in the last decade have an indeterminate growth type, and lower branching and vegetative growth (Procópio et al., 2013; Zanon et al., 2015; Werner et al., 2016). Recent studies have shown that despite the morphophysiological changes, currently used cultivars present a high plasticity; hence, yield per area varies little with significant alterations in plant density and row spacing (Balbinot Junior et al., 2016; Petter et al., 2016).

Soybeans can compensate a lower density by emitting large amounts of branches or increasing branch and stem growth (Ferreira et al., 2016). However, there is a lack of information on the contribution of branch number per plant,

grain production per branch, yield components separately evaluated from branches and stems on crop phenotypic plasticity, mainly for modern cultivars. Thus, this research was based on the following scientific questions: What are the most contributing variables related to soybean branches or stems to grain production per plant? Does it vary with row spacing?

Given the above, the objective of this study was to evaluate the contribution of the branch and soybean stem components to grain production per plant at different plant spatial arrangements using two cultivars with indeterminate growth type.

Results and Discussion

Stepwise regression

In both cropping seasons (2013/14 and 2014/15), water availability during vegetative growth was adequate but with a few water deficit events during the grain-filling period (Figure 2). However, in the 2013/14, the duration of the water deficit was higher, which limited the grain yield. The stepwise regression analysis indicated the number of pods per plant from branches (NPPLB) as the variable with the highest predictive capacity for grain production per plant (PROD) in four spatial arrangements (Tables 1 and 2, and Figures 3 and 4). In all situations, NPPLB was able to explain more than 82% of the variation in PROD.

Besides NPPLB, the adjusted coefficient of determination (R^2) was increased with the insertion of number of pods per plant from stems (NPPLS), thousand-grain mass from stems (TGMS), and number of grains per pod from branches (NGPB), demonstrating a relevant grain production in stems at all row spacing arrangements. In this sense, for both cropping seasons and the four row spacing, grain yield variations in stems contributed to the phenotypic plasticity of soybeans. The number of branches per plant (NBPL) was not selected in the stepwise model for PROD prediction, except for twin row spacing in the 2014/15 cropping season. Soybeans can produce a large number of branches, but with small sizes and low grain yield, or even unproductive ones (Balbinot Junior et al., 2015a). Generally, one of the main variables for phenotypic plasticity evaluations of a cultivar is NBPL (Werner et al., 2016). Contrarily, this research clearly indicates this variable as less relevant than NPPLB.

Based on the angular coefficients of the adjusted models, PROD increase rate was $0.233 \text{ g plant}^{-1}$ due to the increment of one pod per plant from branches for the four spatial sets in the 2013/14 cropping season (Figure 3). In the 2014/15 cropping season, it was 0.426 (Figure 4). This result might have occurred because of a deeper drought during the 2013/14 in the grain filling phase (Figure 2), reducing the effect of a higher NPPLB on production per plant. Therefore, under better conditions (2014/15), soybeans were more able to compensate the lower plant densities by increasing the grain production per plant. Similarly, De Bruin and Pedersen (2008) also observed soybean higher capacity to increase grain yield per plant at low densities when water was available during the crop development cycle. Thus, we may assume that under favorable environments, soybean-sowing density can be reduced without changing grain yield; however, with a substantial decrease in cost of seeds as discussed by Thompson et al. (2015).

Principal component analysis (PCA)

Through principal component analysis (PCA), NPPLB, NPPLS, NBPL, and grain production from branches (PGB) showed to be the variables most associated with PROD (Figures 5 and 6), once vectors were long and close to PROD. In contrast, NGPB and number of grains per pod from stems (NGPS) had small contribution, being far from the PROD. In general, the vector of TGMS and thousand-grain mass from branches (TGMB) were opposite to PROD, except for twin row spacing in the 2013/14 cropping season. Ferreira et al. (2016) verified a significant reduction in thousand-grain mass by decreased sowing densities. They also noted an increase in the percentage of grains from branches, which might have decreased demanded force to stems. These results are in agreement with those of Bellaloui et al. (2015), which showed decreases in protein, sucrose, glucose, raffinose, boron, and phosphorus contents in grains as a function of sowing density reductions. Kumagai et al. (2015) also found that number of pods per plant (stems plus branches) as the main indicator of phenotypic plasticity in soybean cultivars. In this context, we could verify that the mechanisms of phenotypic plasticity in modern cultivars with indeterminate growth type, compact plant architecture and early cycle were the same for the four row spacing and both cropping seasons, even presenting different water availability during crop development cycle.

Materials and Methods

Experimental area

Four experiments were conducted in Londrina, Paraná, Brazil ($23^{\circ}11'S$, $51^{\circ}11' W$ and 620 m above sea level, CfaKöpen-Geiger climate, Rhodic Eutrudox soil type, USDA) during the 2013/14 and 2014/15 cropping season. Each experiment was carried out with one of the following spacing between the rows: Experiment 1= 0.2 m (narrow row); Experiment 2= 0.5 m (traditional); Experiment 3= 0.5 m (crossed rows) and Experiment 4= 0.2/0.8 m (twin rows) (Figure 1). The soil of the experimental area had the following attributes in the 0-20 cm layer: 21.4 g dm^{-3} of organic carbon, 4.9 pH in CaCl_2 , 8.6 mg dm^{-3} of P (Mehlich 1), $0.55 \text{ cmolc dm}^{-3}$ exchangeable K, $3.7 \text{ cmolc dm}^{-3}$ exchangeable Ca and $1.4 \text{ cmolc dm}^{-3}$ exchangeable Mg. The sequential climatological water balance (SCWB) of Thornthwaite and Mather (1955) at the two cropping season is presented in Figure 2. For SCWB determination, the reference evapotranspiration (ET_o) was calculated during the experimental period by Penman-Monteith and transformed into soybean evapotranspiration (ET_c = ET_o x K_c). The available soil water capacity for the SCWB calculation was 75 mm and the adopted K_c was 0.35.

Treatments and experimental design

The experimental design was a randomized complete block with three replicates. In each experiment, we evaluated two soybean cultivars (BMX Potência RR and BRS 359 RR) and three sowing rates (150, 300, and 450 thousand seeds ha^{-1}). Both BMX Potência RR and BRS 359 RR presented an indeterminate growth type and relative maturity group 6.7 and 6.0, respectively. The recommended sowing density is

Table 1. Modeling and adjusted coefficients of determination (R_a^2) calculated by stepwise analysis, considering grain yield per plant of soybean (g) (PROD) as a dependent variable, in four row spacing. Londrina, Paraná state, Brazil, 2013/14 cropping season.

Adjusted models	R_a^2
0.20 m (narrow row)	
PROD=4.19**+0.204 NPPLB**	0.847
PROD=-8.56**+0.193 NPPLB**+5.698 NGPB**	0.933
PROD=-13.51**+0.202 NPPLB**+5.399NGPB**+5.399 TGMS**	0.955
0.50 m (traditional)	
PROD=3.88**+0.235 NPPLB**	0.893
PROD=0.116*+0.206 NPPLB**+0.189 NPPLS**	0.949
PROD=-3.80*+0.210 NPPLB**+0.236 NPPLS**+1.303NGPB**	0.966
0.50 m (crossed rows)	
PROD=3.66**+0.237NPPLB**	0.828
PROD=-0.529ns+0.205 NPPLB**+0.223NPPLS**	0.971
PROD=-3.51*+0.200 NPPLB**+0.234 NPPLS**+1.271 NGPB*	0.977
(0.20/0.80 m) (twin rows)	
PROD=2.93**+0.257 NPPLB**	0.921
PROD=0.291ns+0.227 NPPLB**+0.162NPPLS**	0.947
PROD=-5.21*+0.211 NPPLB**+0.172 NPPLS**+0.059TGMS*	0.963

NPPLB= Number of pods per plant from branches, NGPB = Number of grains per pod from branches, NPPLS= Number of pods per plant from stems, TGMS= Thousand grain mass from stems e NGPB=Number of grains per pod from branches. ** Adjusted Coefficients of Determination (R_a^2)($p \leq 0.01$)and * ($p \leq 0.05$); ns Non-significant coefficients.



Fig 1. Row spacing evaluated: A: 0.2 m (narrow row); B: 0.5 m (traditional); C: 0.5 m (crossed rows) and D: 0.2/0.8 m (twin rows). Londrina, Paraná state, Brazil.

Table 2. Modeling and adjusted coefficients of determination (R_a^2) calculated by stepwise analysis, considering grain yield per plant of soybean (g) (PROD) as a dependent variable, in four row spacing. Londrina, Paraná state, Brazil, 2014/15 cropping season.

Adjusted models	R_a^2
0.20 m (narrow row)	
PROD=6.39**+0.455 NPPLB**	0.875
PROD=4.36**+0.357 NPPLB**+0.131 NPPLS ns	0.888
PROD=-12.66**+0.296 NPPLB**+0.376 NPPLS**+0.080 TGMS**	0.945
0.50 m (traditional)	
PROD=6.59**+0.423NPPLB**	0.867
PROD=-0.57ns+0.432 NPPLB**+0.045TGMS*	0.900
PROD=-14.84**+0.333 NPPLB**+0.387NPPLS**+0.095TGMS**	0.952
0.50 m (crossed rows)	
PROD=7.85**+0.417NPPLB**	0.877
PROD=0.297ns+0.441 NPPLB**+0.035 TGMS**	0.927
PROD=-2.40ns+0.397 NPPLB**+0.159 NPPLS**+0.043TGMS**	0.946
(0.20/0.80 m) (twin rows)	
PROD=6.25**+0.409NPPLB**	0.885
PROD=5.28**+1.04 NPPLB**+0.298NBP**	0.911

NPPLB= Number of pods per plant from branches, NPPLS= Number of pods per plant from stems, TGMS= Thousand grain mass from stems and NBP= Number of branches per plant. ** Adjusted Coefficients of Determination (R_a^2)($p \leq 0.01$)and * ($p \leq 0.05$); ns Non-significant coefficients.

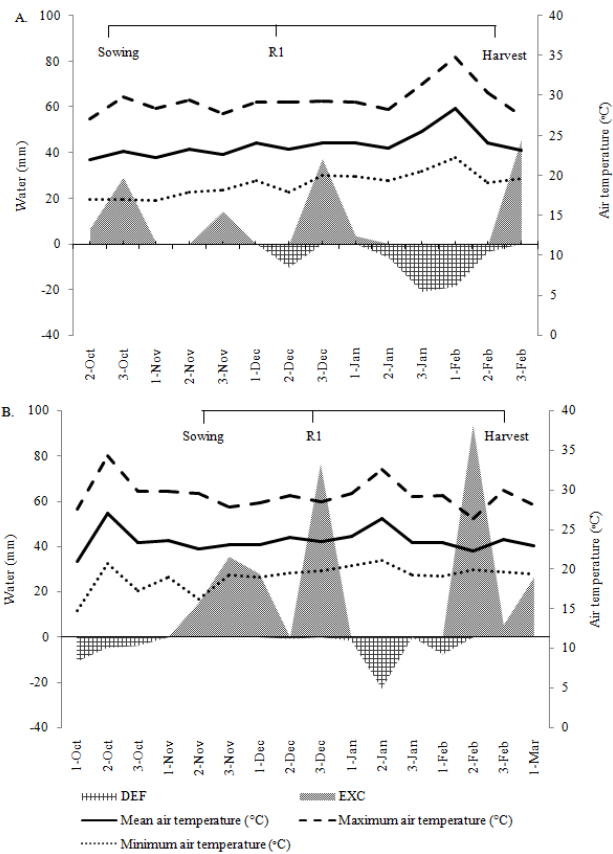


Fig 2. Sequential climatological water balance (mm) of Thornthwaite and Mather during the soybean development cycle. Londrina, Paraná state, Brazil, 2013/14 (A) and 2014/15 (B) cropping seasons.

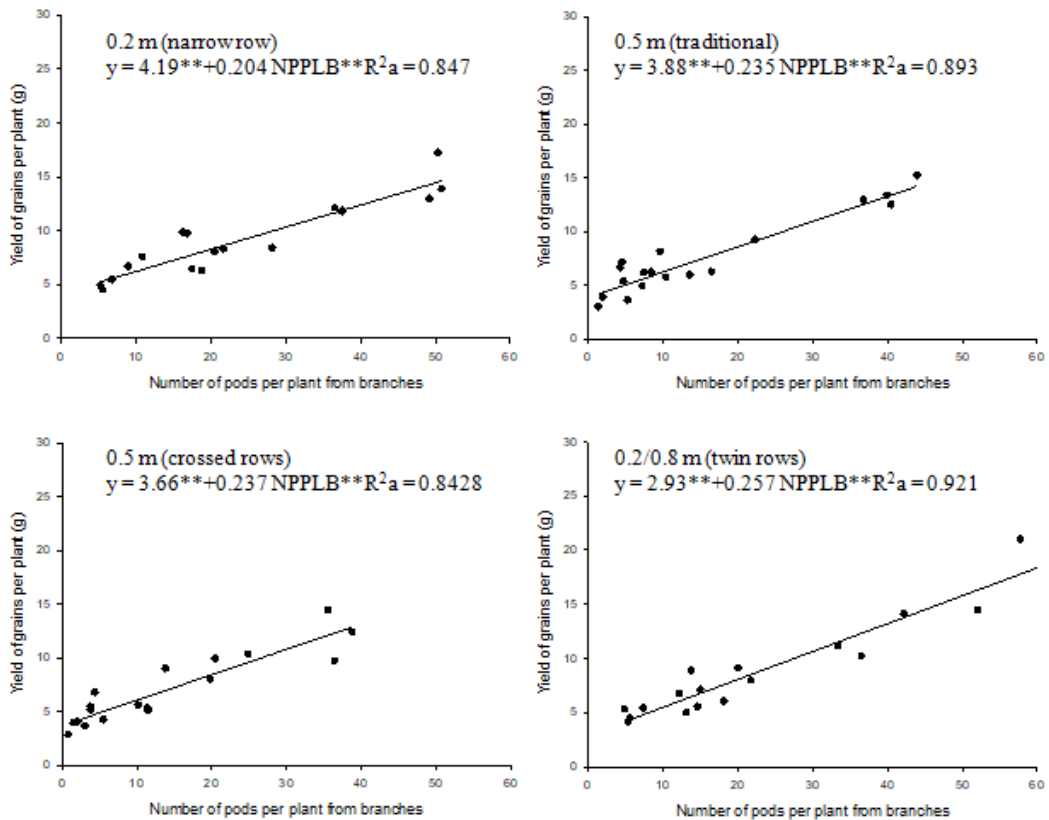


Fig 3. Relationship between the number of pods per plant from branches and the grain yield per plant in four row spacing. Londrina, Paraná state, Brazil, 2013/14 cropping season.

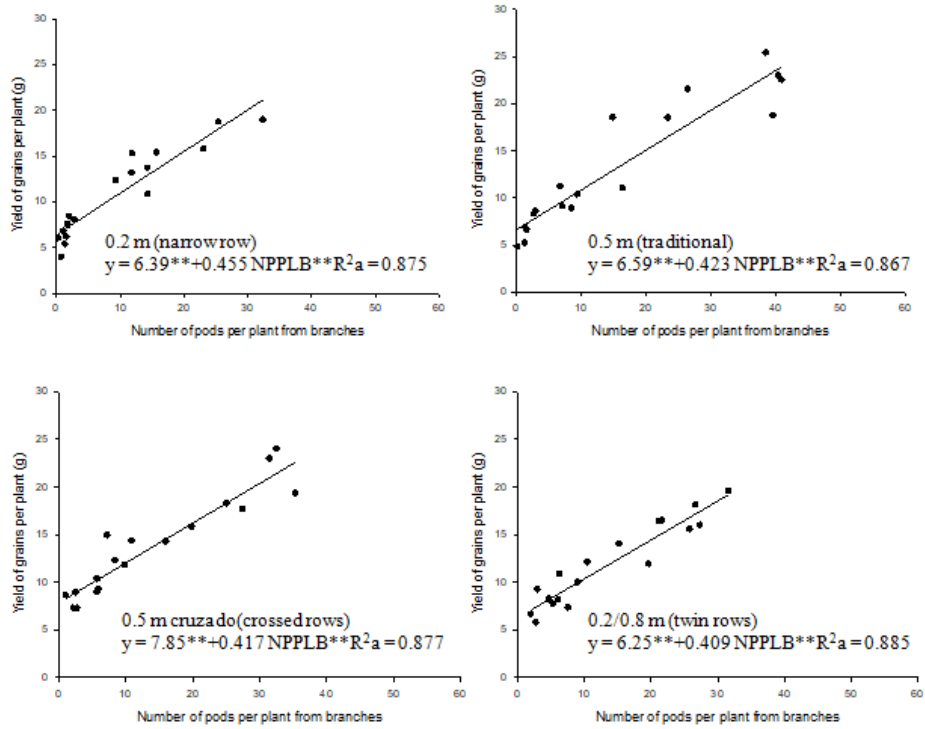


Fig 4. Relationship between the number of pods per plant from branches and the grain yield per plant in four row spacing. Londrina, Paraná state, Brazil, 2014/15 cropping season.

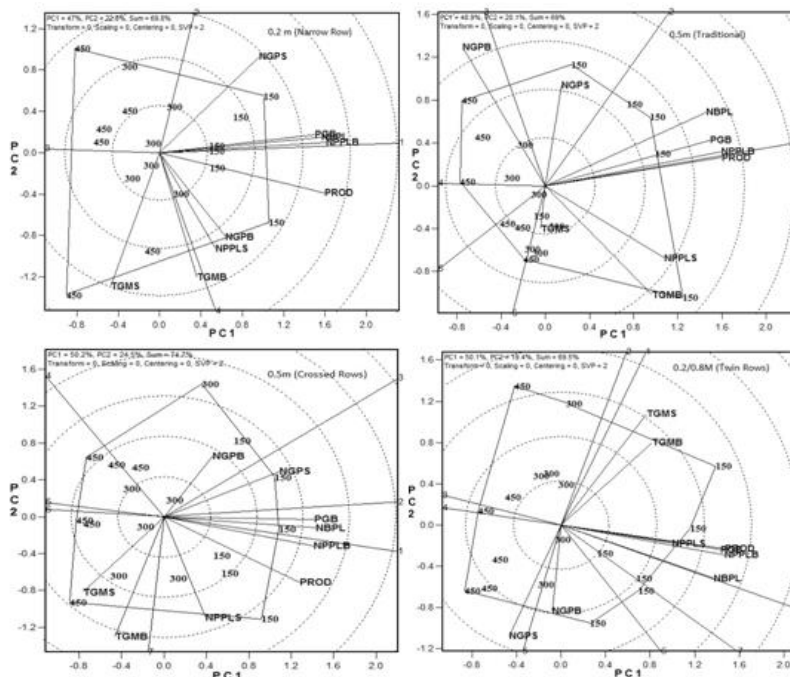


Fig 5. GGE biplot vectors in four row spacing. The independent variables is the number of branches per plant (NBP), number of pods per plant from branches (NPPLB), number of pods per plant from stem (NPPLS), number of grains per pod from branches (NGPB), number of grains per pod from stems (NGPS), thousand-grain mass from branches (TGMB), thousand-grain mass from stems (TGMS) and percentage of grain yield from branches (PGB) and the dependent variable is the production of grains per plant (PROD). The numbers in the figure refer to the evaluated densities: 150, 300 and 450 plants ha⁻¹. Londrina, Paraná state, Brazil, 2013/14 cropping season.

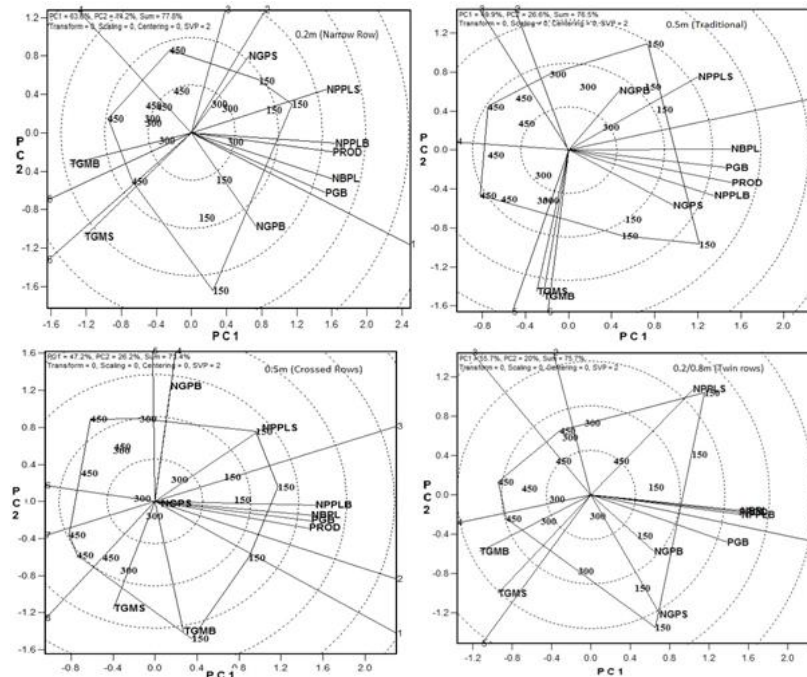


Fig 6. GGE biplot vectors in four row spacing. The independent variables is the number of branches per plant (NBPL), number of pods per plant from branches (NPPLB), number of pods per plant from stem (NPPLS), number of grains per pod from branches (NGPGB), number of grains per pod from stems (NGPS), thousand-grain mass from branches (TGMB), thousand-grain mass from stems (TGMS) and percentage of grain yield from branches (PGB) and the dependent variable is the production of grains per plant (PROD). The numbers in the figure refer to the evaluated densities: 150, 300 and 450 plants ha⁻¹. Londrina, Paraná state, Brazil, 2014/15 cropping season.

between 265,000 to 310,000 plants ha⁻¹ for BMX RR Potência, and from 220,000 to 265,000 ha⁻¹ plants for BRS 359 RR. The plots measured 10 m in length and 5 m in width, totaling 50 m². The useful area used was 24 m² (8 m long and 3 m wide). The two seeding dates were October 23, 2013; and November 11, 2014. On the sowing day, seeds were treated with Vitavax-Thiran 200SC® (Carboxin + Thiram - 150 mL 50 kg⁻¹ of seed) and Gelfix 5® liquid inoculant (100 ML 50 kg⁻¹ of seeds). Soil fertilization consisted of 125 kg ha⁻¹ triple superphosphate (41% of P₂O₅) and 250 kg ha⁻¹ potassium chloride (50% of K₂O) applied ten days prior to sowing.

Traits measured

At harvest, 20 plants per plot were sampled for the following evaluations: grain production per plant (PROD), a dependent variable representing soybean phenotypic plasticity, number of branches per plant (NBPL), number of pods per plant from branches (NPPLB) and from stems (NPPLS), number of grains per pod from branches (NGPGB) and from stems (NGPS), thousand-grain mass from branches (TGMB) and from stems (TGMS), and grain production from branches in % (PGB).

Statistical analysis

Normality, independence of residues, variance homogeneity of treatments, and non-additivity of the model were verified. A stepwise regression analysis was performed to determine models with a higher predictive capacity for production per

plant. In addition, Pearson correlation analysis was performed among the evaluated variables. The regression and correlation analyses were performed through the SAS 9.2 software.

After detecting a significant Pearson correlation (p≤0.05) between the variables and standardizing them for a dimensionless scale, the most relevant independent variables were defined in the determination of production by plant, by principal component analysis (PCA), using the GGEbiplot program. The standardization helped to avoid the variables measured with the largest variances dominate the others. The polygonal biplot with concentric circles was based on the Singular Value Decomposition (SVD). By SVD, the matrix is decomposed into singular values, column eigenvectors and eigenvector lines. The singular value of the matrix is a diagonal matrix. The biplot is formed with the main component scores (CP1) in the abscissa and ordinate scores (CP2) for each treatment and each variable (Yan and Rajcan, 2002) and the model may be expressed by the following equation:

$$\frac{T_{ij} - \bar{T}_j}{s_j} = \lambda_1 \phi_{i1} \tau_{j1} + \lambda_2 \phi_{i2} \tau_{j2} + \varepsilon_{ij}$$

T_{ij} mean value of the densities i for the variable j,
 \bar{T}_j mean value of the variable j on the general mean of the densities,
 λ_1 e λ_2 singular values for the components CP1 and CP2, s_j standard deviation of the variables j between the mean densities, ϕ_{i1} e ϕ_{i2} scores of the main components CP1 and CP2, respectively, for the

density i , τ_{j1} e τ_{j2} scores CP1 and CP2, associated with variables j , ε_{ij} model residue associated with densities i in variable j .

Conclusion

The number of pods per plant from branches was the most determinant variable for the phenotypic plasticity in modern cultivars of soybeans, with indeterminate growth habit regardless of the spatial arrangement. The principal component analysis indicated the number of pods per plant from branches and stems, the number of branches per plant, and the percentage of grain production from branches as variables strongly associated with the phenotypic plasticity of modern cultivars of soybeans. The number of grains per pod and the thousand-grain mass from branches and stems had no significant contribution to the plasticity of soybeans, regardless of the spatial arrangement.

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