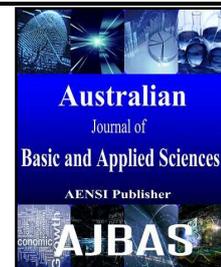




## AUSTRALIAN JOURNAL OF BASIC AND APPLIED SCIENCES

ISSN:1991-8178 EISSN: 2309-8414  
Journal home page: www.ajbasweb.com



### Path analysis and Annicchiarico method applied in relation to protein in corn grains

<sup>1</sup>Ivan Ricardo Carvalho, <sup>2</sup>Maicon Nardino, <sup>1</sup>Alan Junior de Pelegrin, <sup>1</sup>Mauricio Ferrari, <sup>3</sup>Gustavo Henrique Demari, <sup>3</sup>Vinicius Jardel Szarecki, <sup>4</sup>Mauricio Horbach Barbosa, <sup>5</sup>Velci Queiróz de Souza

<sup>1</sup>Federal University of Pelotas – Plant Breeding Department, Campus Universitário, s/n, 96160-000, Capão do Leão, Rio Grande do Sul, Brazil.

<sup>2</sup>Federal University of Pelotas – Physics and Mathematics Institute, Campus Universitário, s/n, 96160-000, Capão do Leão, Rio Grande do Sul, Brazil.

<sup>3</sup>Federal University of Pelotas – Department of Seed Science and Technology, Campus Universitário, s/n, 96160-000, Capão do Leão, Rio Grande do Sul, Brazil.

<sup>4</sup>Federal University of Santa Maria, Campus Frederico Westphalen - Agronomy Department, Linha 7 de Setembro, s/n, BR 386 Km 40, 98400-000, Frederico Westphalen, Rio Grande do Sul, Brazil.

<sup>5</sup>Federal University of Pampa, Campus Dom Pedrito – Rua 21 de Abril, Bairro São Gregório, n° 80, 96450-000, Dom Pedrito, Rio Grande do Sul, Brazil.

**Address For Correspondence:**

Ivan Ricardo Carvalho, Federal University of Pelotas – Plant Breeding Department, Campus Universitário, s/n, 96160-000, Capão do Leão, Rio Grande do Sul, Brazil.  
E-mail: irc.carvalho@gmail.com

**ARTICLE INFO**

**Article history:**

Received 13 April 2016

Accepted 21 May 2016

Published 28 May 2016

**Keywords:**

Interrelations, phenotypic stability, nutritional quality, biometrics.

**ABSTRACT**

To obtainment of nutritionally superior genotypes on protein level is necessary, this study aimed to identify linear associations, cause and effect between traits and crude protein stability of corn grains in four different environments Annicchiarico method. The experiment was conducted in harvest the 2013/2014, in four growing environments in the state of Rio Grande do Sul, Brazil. As follows: Campos Borges – RS, Fortaleza dos Valos - RS, Santa Rosa – RS, Tenente Portela - RS. The experimental design was of randomized blocks arranged in a factorial with four crop environments x 15 single corn hybrid genotypes, arranged in three repetitions. Traits measured were: cob diameter (CD), cob length (CL), cob matter (CM), cob insertion height (CIH), number of cob grain rows (NCGR), plant height (PH), number of grains per cob row (NGCR), prolificacy (PRO), thousand-grain weight (TGW), grain yield (YIELD) and crude protein percentage (CPP). Plant height, thousand-grain weight and grain yield increase reduces corn grain protein content. Santa Rosa - RS and Tenente Portela – RS are considered favorable environments for crude protein. Annicchiarico method showed that in general, grain crude protein percentage was not stable and predictable in the four tested environments. LG 6304 YG® and FORMULA TL® genotypes stood out as stable for this traits in the four environments.

**INTRODUCTION**

Corn (*Zea mays* L.) is used for different purposes, including human food, animal feed and industrialized products. Around 70% of its world production is intended for animal nutrition (Paes, 2006). Corn grain composition has 62.48% starch, 8.26% protein, 3.61% lipids and 1.27% mineral material (Rostagno *et al.*, 2011). Among corn grain constituents, protein content has great influence on animal nutrition, as proteins are made of amino acids, which are vital to the growth and development of body structures such as muscles, nervous system and immune system in animals (Toledo *et al.*, 2004).

Grain crude protein grain assumes complex genetic control due to the expression of various genes,

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**To Cite This Article:** Ivan Ricardo Carvalho, Maicon Nardino, Alan Junior de Pelegrin, Mauricio Ferrari, Gustavo Henrique Demari, Vinicius Jardel Szarecki, Mauricio Horbach Barbosa, Velci Queiróz de Souza., Path analysis and Annicchiarico method applied in relation to protein in corn grains. *Aust. J. Basic & Appl. Sci.*, 10(9): 300-306, 2016

culminating in increased crop environment effect (Reyes Moreno and Parede Lopes, 1993). Research determined that protein content is highly influenced by environmental factors, such as water availability, temperature, edaphoclimatic conditions and nutrient availability, especially of nitrogen (Buratto *et al.*, 2009).

Currently, corn breeding shows grain yield quantitative increase in many cases (Lima and Bellaver, 1999). However, some of the consumer market niches prioritize higher grain nutritional value. Therefore, obtainment of nutritionally superior genotypes on protein level is necessary. Thus, breeding programs aimed at obtaining genotypes with higher grain protein ratios highlight the need to understand what the determinant traits for crude protein increase. With the purpose of assisting in the selection of which traits enable the indirect selection process for superior genotypes, Pearson linear correlation analysis is used to define the tendency of linear associations between trait pairs. However, its direct interpretation may lead to interpretation errors, making the split of linear relations in cause and effect associations through path analysis crucial (Wight, 1921). Which allows for determining the explanatory traits direct and indirect effects on the dependent trait (Cruz *et al.*, 2014). Given crude protein importance and the need to reveal crop environment effects on this trait. Annicchiarico method uses how genotype stability in relation to trait averages for each environment tested. Thus, the results are based on the estimation of a confidence index for each genotype, allowing for inferring which of these genotypes are superior and which environments are favorable or unfavorable to the interest trait (Cruz *et al.*, 2014). Due to lack of research qualitatively addressing corn grain and traits interrelations with crude protein, in addition to trait stability by Annicchiarico method in relation to crop environment variations.

The aim of this study was to identify linear and cause and effect associations between traits and crude protein stability of corn grains grown in four different environments by Annicchiarico method.

## MATERIAL AND METHODS

The experiment was conducted in the 2013/2014 season in four growing environments in the state of Rio Grande do Sul, Brazil. As follows: Campos Borges - RS located in 28°55'36"S and 53°01'40"W coordinates, with an altitude of 513 m and a dark-red Latosol; Fortaleza dos Valos - RS located in 28°47'50"S and 53°13'22"W coordinates, with an altitude of 406m and a dark-red Latosol; Santa Rosa - RS located in 27°52'16"S and 54°28'55"W coordinates, with an altitude of 268 m and a dystrophic Red Latosol; Tenente Portela - RS, located in 27°23'31"S and 53°46'50"W coordinates, with an altitude of 420 m in atypical ferric aluminum Red Latosol (Embrapa, 2006). Climate for all environments was characterized by Köppen as subtropical *Cfa* (Moreno, 1961).

The experimental design was of randomized blocks arranged in a factorial with four crop environments x 15 single corn hybrid genotypes, arranged in three repetitions. Genotypes were, as follows: 2A106, 30F53, P2530, ADV9434PRO<sup>®</sup>, AS1656PRO<sup>®</sup>, DKB245PRO<sup>®</sup>, LG6304YG<sup>®</sup>, FORMULA TL<sup>®</sup>, CELERON TL<sup>®</sup>, P30F53Hx<sup>®</sup>, P3646Hx<sup>®</sup>, P1630Hx<sup>®</sup>, MAXIMUS VIP3<sup>®</sup>, DEFENDER VIP<sup>®</sup> and IMPACTP VIP3<sup>®</sup>.

The experimental units consisted of four rows with five meters long with 0.5 m spaced, totaling 10 m<sup>2</sup>. Direct seeding system was used in all environments, with a population density of 80000 plants per hectare. Basal dressing with 300 kg ha<sup>-1</sup> de NPK in the 10-20-20 formulation was used; 135 kg ha<sup>-1</sup> nitrogen in starch form was used as topdressing, applied in V<sub>4</sub> and V<sub>6</sub> vegetative stages (Fancelli and Dourado Neto, 2000). Weeds, insect pests and disease management were carried out preventively, in order to reduce interference in the experiment results.

Interest traits were measured in the useful area of each experimental unit, which consisted of two central rows where 0.5 m from each extremity was not used. Measured traits were, as follows: cob diameter (CD), expressed in mm; cob length (CL), expressed in cm; cob matter (CM), expressed in g; cob insertion height (CIH), expressed in m; number of cob grain rows (NFIL), expressed in units; plant height (PH), expressed in m; number of grains per cob row (NGCR), expressed in units; prolificacy (PRO), expressed in units; thousand-grain weight (MMG), expressed in g; grain yield (YIELD), expressed in kg ha<sup>-1</sup>, following the methodology proposed by Carvalho *et al.* (2014) and crude protein percentage (CPP) measured by the methodology proposed by Nogueira and Souza (2005).

Data were submitted to individual variance analysis for each crop environment, in order to verify the assumptions *a priori*. Residues univariate normality diagnosis (Toebe and Carnelutti Filho, 2013) was conducted by Shapiro-Wilk test (Shapiro and Wilk, 1965), and variance homogeneity was conducted by Bartlett test (Steel *et al.*, 1997). Subsequently, joint analysis was conducted to identify interaction presence between crop environments x single corn hybrid genotypes at 5% probability. *Pearson* linear correlation, aimed at highlighting linear association trend between traits (Carvalho *et al.*, 2004), was conducted afterwards. Through phenotypic linear correlation matrix, multicollinearity diagnosis was conducted by the number of conditions (NC) of the matrix (Cruz *et al.*, 2012). Phenotypic path analysis was conducted to breakup cause and effect interrelations by setting the crude protein percentage trait as dependent and other traits as explanatory (Cruz *et al.*, 2014). Phenotypic linear correlation coefficients matrix was subjected to multicollinearity diagnosis, where the number of matrix conditions (NC) was tested. Preliminary diagnosis showed moderate to strong co linearity

(NC = 100 <a <1000). Annicchiarico method (1992) was applied to crude protein percentage trait (CPP) character, and followed the methodology proposed by Cruz *et al.* (2014). Analyses were conducted using the Genes statistical software (Cruz, 2013).

## RESULTS AND DISCUSSION

Analysis of variance was significant ( $p < 0.05$ ) in crop environments x corn genotypes interaction for traits: crude protein percentage (CPP), cob diameter (CD), cob insertion height (CIH) and grain yield (YIELD). Interaction absence was observed for cob length (CL), cob matter (CM), number of cob grain rows (NCGR), number of grains per cob row (NGCR), plant height (PH), prolificacy (PRO) and thousand-grain weight (TGW) (Table 1).

**Table 1:** Summary of the analysis of variance for 15 genotypes (G) of hybrid maize and four growing environments (E).

	Square medium										
	CPP	CD	CL	CM	NCGR	NGCR	CIH	PH	PRO	TGW	YIELD
environments(E)	69.5*	605.3*	100.4*	87072.2*	9.5*	1600.0*	5466.4*	52916.3*	0.6*	87174.5*	269672458.8*
genotypes (G)	12.7*	20.6*	6.3*	1416.8	10.1*	25.9*	595.8*	783.3*	0.1	5133.3*	21161069.3*
E x G	10.5*	9.2*	2.8	1146.9	1.9	14.2	294.6*	306.0	0.0	1133.2	12216451.8*
Block	0.7	22.8*	1.6	1207.4	0.4	11.1	917.2*	810.0	0.0	4050.2*	820.7
Residue	0.4	5.7	2.5	835.5	1.5	13.7	152.2	276.2	0.1	972.1	20968.8
VC (%)	0.8	5.2	9.7	19.4	7.8	12.0	12.1	8.8	24.6	11.2	5.4

\* Significant at 5% by F test.

CD:cob diameter; CL:cob length; CM:cob matter; CIH:cob insertion height; NCGR:number of cob grain rows; PH:plant height; NGCR:number of grains per cob row; PRO:prolificacy; TGW:thousand-grain weight; YIELD:grain yield; CPP:crude protein percentage.

Regarding variation coefficients obtained for traits measured in the experiment, it was observed range from 0.82 to 24.64% was observed. According to the classification by Pimentel Gomes (2000), coefficients of variation (VC) may be considered low (<10%) with high accuracy, medium (10 to 20%) with good accuracy, high (20 to 30%) with low accuracy and very high (>30%) with low accuracy. Thus, VC obtained in this study show reliable results and adequate experimental accuracy.

Regarding Pearson linear correlation analysis, 55 associations were obtained and 33 pairs were significant at 5% probability (Table 2). For correlation magnitude inferences, the classification by Carvalho *et al.* (2004) was adopted. For crude protein percentage (CPP) trait, low and positive trend ( $r = 0.18$ ) with PRO and intermediate and negative trend ( $r = -0.36$ ) with TGM were observed, which were both significant. Indicating that more prolific plants tend to produced grains with lower matter, In turn, grain protein ratio is increased, which is justified through starchy carbohydrates and protein synthesis in the endosperm, where larger grains tend to accumulate higher starchy than protein ratios. Starchy carbohydrates accumulation in grains occurs due to the sugar content stored up to 46 days after anthesis. However, grain protein accumulation occurs 40 days after anthesis (Magalhães and Souza, 2011).

Cob diameter (CD) shows intermediate to high and positive trends with CL ( $r = 0.61$ ), CM ( $r = 0.74$ ), CIH ( $r = 0.42$ ), NCGR ( $r = 0.37$ ), PH ( $r = 0.67$ ), NGCR ( $r = 0.74$ ) and YIELD ( $r = 0.55$ ), and low and positive trends ( $r = 0.26$ ) with TGW. Thus, corn genotypes with greater height and larger cobs tend to increase the number of rows and grains per cob influencing grain matter and crop yield. During the vegetative period, corn stores its assimilates into the stem (Doebley,2004). When the reproductive period starts, assimilates are directed to reproductive structures. Thus, photosynthetic efficiency, assimilates accumulation, remobilization capacity and their direction to the cob directly influence grain filling and dimensions. This dynamic is intrinsic to each genotype, and is highly influenced by the environment (Karam *et al.*, 2010). Cob length (CL) showed high and positive trend with CM ( $r = 0.73$ ) and NGCR ( $r = 0.72$ ) and intermediate and positive trend with CIH ( $r = 0.43$ ), PH ( $r = 0.52$ ), TGW ( $r = 0.43$ ) and YIELD ( $r = 0.37$ ). Therefore, grains with higher longitudinal extent tend to have more grains and higher cob matter and grain matter per cob, reflecting in higher grain yield.

Cob matter (CB) shows high positive association with NGCR ( $r = 0.78$ ), TGW ( $r = 0.64$ ) and YIELD ( $r = 0.64$ ), intermediate and positive association with PH ( $r = 0.45$ ) and low and positive association with CIH ( $r = 0.29$ ). Thus, it is evident that cobs with higher matter and to increase the number of grains per row and thousand-grain weight, and the association of these characters provides grain yield increases. Bortolini *et al.* (2001), highlighted the number of cobs per plant, number of grains per cob and grain yield as traits responsible for corn yield.

**Table 2:** Estimates of Pearson correlation coefficients for 11 agronomically important traits in 15 simple hybrids of corn and four growing environments.

	CPP	CD	CL	CM	CIH	NCGR	PH	NGCR	PRO	TGW	YIELD
CPP	-	0.09	0.01	-0.13	0.07	0.13	0.14	0.08	0.18*	-0.36*	-0.04
CD		-	0.61*	0.74*	0.42*	0.37*	0.67*	0.74*	0.12	0.26*	0.55*
CL			-	0.73*	0.43*	0.01	0.52*	0.72*	0.02	0.43*	0.37*
CM				-	0.29*	0.12	0.45*	0.78*	-0.07	0.64*	0.64*
CIH					-	0.06	0.64*	0.34*	0.21*	0.06	0.12
NCGR						-	0.15*	0.11	0.15	-0.30*	-0.01
PH							-	0.63*	0.17*	-0.01	0.44*
NGCR								-	0.02	0.23*	0.59*
PRO									-	-0.25*	-0.14
TGW										-	0.43*
YIELD											-

\* Linear correlation coefficients of *Pearson* (n = 180) significant at 5% probability.

(<sup>1</sup>)CD:cob diameter; CL:cob length; CM:cob matter; CIH:cob insertion height; NCGR:number of cob grain rows; PH:plant height; NGCR:number of grains per cob row; PRO:prolificacy; TGW:thousand-grain weight; YIELD:grain yield; CPP:crude protein percentage.

Cob insertion height (CIH) showed high and positive trend with PH ( $r = 0.64$ ), intermediate and positive trend with NGCR ( $r = 0.34$ ) and low and positive trend with PRO ( $r = 0.21$ ). Plants with higher cob insertion height tend to have greater stature and cobs with more grains per row and prolific plants. Santos *et al.* (2002), when evaluating corn hybrids agronomic performance, showed high positive correlation between plant height and cob insertion.

The number of cob rows (NCR) showed low and positive trend with PH ( $r = 0.15$ ) and low and negative trend with TGW ( $r = -0.30$ ). Thereby, cobs with more rows occur due to higher plants and lower grain matter. Plant height (PH) has high and positive trend with NGCR ( $r = 0.63$ ) intermediate and positive trend with YIELD ( $r = 0.44$ ) and low and positive trend with PRO ( $r = 0.17$ ). Linear associations indicate that higher plants tend to increase cob dimensions and grain yield. Research by Revolti *et al.* (2014), showed high positive correlation between plant height and grain yield for corn, regardless of nitrogen management.

According to Kleinpaul *et al.* (2014), corn hybrids with early cycle showed positive correlation between plant height, cob index and grain yield. The number of grains per cob row (NGCR) showed intermediate and positive trend with the YIELD ( $r = 0.59$ ) and low and positive trend with TGW ( $r = 0.23$ ). Thus, smaller cobs tend to increase grain matter and grain yield. The prolificacy (PRO) showed low and negative trend ( $r = -0.25$ ) with TGW, indicating that more prolific plants tend to develop grains with lower matter. These results are due to changes in the  $r$  source/drain ratio. Plants are supported by an order of its grain formation and development when developing more cobs per plant. Thus, the upper cob tends to form a larger number of grains, and more assimilates are needed to compose its structures. However, when there are photosynthetic and assimilate restrictions, the secondary cob tends to reduce grain number and matter (Magalhães *et al.*, 2002).

Thousand-grain weight (TGW) showed intermediate and positive trend with YIELD, Thus, corn genotypes with higher grain matter and to increase grain yield. Those results corroborate with Ribeiro (2012). Relations between yield components and corn morphological traits show that grain matter is directly responsible for increasing grain yield (Lopes *et al.*, 2007; Souza *et al.*, 2014; Souza *et al.*, 2015).

Cause and effect phenotypic associations were employed for the four crop environments and 15 corn hybrids jointly, in which crude protein percentage (CPP) was set as the dependent trait and NCGR, NGCR, PH, PRO, TGW and YIELD were set as explanatory traits. In corn breeding programs aimed at increasing nutritional quality, knowledge of crude protein percentage is critical. However, its determination is costly regarding time and financial resources. In this sense, a valid alternative is to use indirect selection to select genotypes with the highest grain protein percentage, and to understand which traits should be considered to guide the selection strategy, through traits with easy measurement, high heritability and great effect to the interest trait (Hartwig *et al.*, 2007).

Number of rows per cob (NRC) trait showed direct intermediate and positive effect to CPP (Table 3). Total correlation was ( $r = 0.604$ ) high and positive, proving the direct effects of NCGR and CPP, showing a cause and effect relation. Sgarbieri (1996) determined that grain protein is in higher ratios contained in the embryo. Thus, it is possible to infer that protein content increase is due to the increase of grains per cob.

The number of grains per cob row (NGCR) showed direct effects of medium magnitude with CPP ( $r = 0.52$ ), indicating that plants with increased NGCR have higher protein content. However, linear correlations point to the opposite signal, indicating that the NGCR variable is influenced by other physiological factors (Falconer, 1996). Plant height (APL) expressed intermediate and negative direct effects to the crude protein percentage (CPP) trait. Indirectly, low and positive effects were observed with NGCR, and low to intermediate negative effects were observed with TGW and YIELD. Results indicated that genotypes with reduced plant height develop cobs with increased length and lower matter and yield. Although increasing grain protein fraction, total correlation is ( $r = -0.806$ ) strong and negative, and proves the direct effects obtained by PH with

CPP.

Prolificacy (PRO) showed negative direct effects on CPP, total correlation ( $r = 0.390$ ) was intermediate to negative due to indirect negative effects. Thousand-grain weight (TGW) showed high and negative direct effect with crude protein percentage (CPP). For Magalhães *et al.* (2002), reserve accumulation mainly occurs in the endosperm during grain development, in which starchy carbohydrates are the main constituents. Secondly, there is the increase in protein content increase. Research showed that starchy carbohydrates and protein accumulation are related, but carbohydrate accumulation occurs over a longer period when compared to protein accumulation; with both occurring between grain anthesis and physiological maturity. Total correlation was ( $r = -0.940$ ) high and negative, showing that there is cause and effect relation between TGW and CPP traits.

Grain yield (YIELD) expressed intermediate and negative direct effect to crude protein percentage (CPP). Alves *et al.* (2015) indicated that corn grain yield increase caused grain protein and amino acid levels decrease. Total correlation was ( $r = -0.380$ ) intermediate and negative, confirming YIELD direct effects with CPP. Determination coefficient ( $R^2$ ) was high 0.98 which indicates that the results were adequately explained by the proposed model and residual effects were low (0.10) with association reliability.

**Table 3:** Estimates of the direct and indirect effects in six phenotypic traits of agronomic interest in the crude protein percentage (CPP) 15 genotypes derived from hybrid maize and four growing environments.

Effects	Exploratory characters					
	NCGA	NGCR	PH	PRO	TGW	YIELD
Direct <sup>(1)</sup>	0.47	0.52	-0.41	-0.58	-0.61	-0.35
Ind. via NCGA	-	-0.17	0.02	0.37	-0.53	-0.08
Ind. via NGCR	-0.19	-	0.13	0.21	-0.04	0.49
Ind. via PH	-0.01	-0.11	-	-0.02	-0.08	-0.40
Ind. via PRO	-0.48	-0.24	-0.03	-	0.44	0.08
Ind. via TGW	0.69	0.05	-0.11	0.46	-	-0.07
Ind. via YIELD	0.06	-0.33	-0.34	0.04	-0.04	-
Total (r)	0.60	-0.20	-0.81	0.39	-0.94	-0.38
Determination coefficient			0.99			
Value of K used in the analysis			0.15			
Effect of the residual variable			0.11			
Determinant of the matrix			0.06			

<sup>(1)</sup>CD:cob diameter; CL:cob length; CM:cob matter; CIH:cob insertion height; NCGR:number of cob grain rows; PH:plant height; NGCR:number of grains per cob row; PRO:prolificacy; TGW:thousand-grain weight; YIELD:grain yield; CPP:crude protein percentage.

Regarding stability analysis, Annicchiarico method was used to identify among 15 corn genotypes in four crop environments, which genotypes demonstrated differential effect to increase grain crude protein. In addition, it was also used to express which environments are favorable or unfavorable to this trait, as corn grain protein and oil contents are quantitative traits (Song *et al.*, 1999) that tend to be controlled by multiple genes and tend to be strongly influenced by crop environment.

Santa Rosa - RS and Tenente Portela- RSEnvironments were favorable to the grain crude protein percentage(CPP) trait. On the other and Campos Borges - RS and Fortaleza dos Vales-RS were characterized as unfavorable (Table 4). Favorable environments for protein in corn grains are those with the best conditions for the expression of this trait and with suitable nutritional conditions, mainly of nitrogen (Ferreira *et al.*, 2001). According to Duarte *et al.* (2005), nitrogen increase in 14.2% results in 19.1% corn grain protein increase. Climate conditions have great influence on corn physiological processes, altitude directly influences air temperature, solar radiation intensity, photosynthetic rate, plant growth and development, water use efficiency, respiratory rates and nutrient absorption, assimilation and translocation by plant structures (Magalhães and Souza, 2011). Studies showed that water availability in the reproductive period expressed direct effects to amino acid synthesis and total protein (Magalhães *et al.*, 2002).

**Table 4:** Confidence indices estimates (Ii) the Annicchiarico method with decomposition Ii general. (Ii) favorable, (Ii) unfavorable and environmental index, for the character percentage of crude protein (CPP) for 15 corn genotypes in four growing environments.

Environments	Average of CPP		Environmental index		Classification *	
Campos Borges-RS	6.33		-0.17		U	
Fortaleza dos Vales-RS	4.80		-1.70		U	
Santa Rosa-RS	7.47		0.97		F	
TenentePortela-RS	7.39		0.89		F	
	General		Unfavorable		Favorable	
Genotypes	Avera	Ii	Averag	Ii	Averag	Ii
2A106	5.52	86.87	5.86	104.27	5.17	69.46
30F53	6.40	101.81	6.78	122.69	6.01	80.93
P2530	6.26	98.25	5.31	99.66	7.20	96.85
ADV9434 PRO®	5.92	85.24	3.16	53.68	8.68	116.81
AS1656 PRO®	7.77	122.72	7.54	137.70	8.00	107.75
DKB245 PRO®	6.23	91.72	4.65	78.14	7.82	105.29
LG6304 YG®	8.10	128.61	7.51	140.31	8.69	116.91

FORMULA TL <sup>®</sup>	8.05	127.01	7.97	144.65	8.13	109.36
CELERON TL <sup>®</sup>	5.94	87.81	4.76	79.69	7.13	95.92
P30F53 Hx <sup>®</sup>	6.97	109.17	6.17	113.78	7.77	104.56
P3646 Hx <sup>®</sup>	5.28	75.96	2.68	45.80	7.89	106.12
P1630 Hx <sup>®</sup>	6.33	96.98	5.24	94.09	7.42	99.87
MAXIMUS VIP3 <sup>®</sup>	6.18	91.27	5.15	85.51	7.21	97.03
DEFENDER VIP <sup>®</sup>	4.74	74.65	4.63	84.07	4.86	65.23
IMPACTP VIP3 <sup>®</sup>	7.80	121.93	6.09	115.94	9.51	127.91

\* Alpha: 0.95; U: Unfavorable; F: Favorable.

Single corn hybrids, when grown in crude protein unfavorable environments, showed that FORMULA TL<sup>®</sup> (7.97%), LG6304 YG<sup>®</sup> (7.51%), AS1656 PRO<sup>®</sup> (7.54%), 30F53 (6.78%), IMPACTO VIP3<sup>®</sup> (6.09%), P30F53 Hx<sup>®</sup> (6.17%) and 2A106 (5.86%) increased the trait. In favorable environments, IMPACTO VIP3<sup>®</sup> (9.51%), LG6304 YG<sup>®</sup> (8.69%), ADV9434 PRO<sup>®</sup> (8.68%), FORMULA TL<sup>®</sup> (8.13%), AS1656 PRO<sup>®</sup> (8.00%) and DKB245 PRO<sup>®</sup> (7.82%) genotypes increased grain crude protein.

LG6304YG<sup>®</sup> and FORMULA TL<sup>®</sup> genotypes showed stability for grain protein trait in unfavorable and favorable environments, with magnitudes of 8.10 and 8.05%, respectively. This response shows that these genotypes are predictable in the response of this trait, and can be indicated as genotypes that are stable and less influenced by environmental characteristics regarding crude protein.

Linear and cause and effect associations joint interpretation, along with Annicchiarico method, allowed for revealing interrelations between traits and defining which of these are determinant for crude protein percentage in corn grains. In addition, associations and Annicchiarico method also showed which environments are favorable or unfavorable and which genotypes are more stable and predictable for this trait.

### Conclusions:

Plant height, thousand-grain weight and grain yield increase reduces corn grain protein content.

Santa Rosa - RS and Tenente Portela - RS are considered favorable environments for crude protein.

Annicchiarico method showed that in general grain crude protein percentage was not stable and predictable in the four tested environments.

LG 6304 YG<sup>®</sup> and FORMULA TL<sup>®</sup> genotypes stood out as stable for this trait in the four environments.

### ACKNOWLEDGEMENTS

The first author acknowledges a fellowship by Universidade de Federal de Santa Maria *Campus* Frederico Westphalen (UFSM) Brazil and Laboratório de Melhoramento Genético e Produção de Plantas (UFSM).

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