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Artigo Científico

Response of maize crop hybrids, with different transgenic events, to inoculation with *Azospirillum brasilense*

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Abstract

The inoculation of maize crop hybrids with *Azospirillum brasilense* is an important alternative to increase grain yield and decrease the production costs of this crop. However, there is a specificity between *A. brasilense* and the hybrid to be inoculated, mainly maize hybrid with transgenic event. Thus, the aim of the study was to evaluate how maize hybrids with different transgenic events respond to inoculation with *A. brasilense*. The study was carried out in Cândói, Paraná State, in two experiments, in a completely randomized design with four replications. The experiment I, in the cropping season 2014/15, was carried out in a 5 x 2 factorial arrangement, with five hybrids (AS1656 PRO II, AS1656 PRO III, AG9045 RR2, AG9045 PRO and AG9045 PRO III), in the presence or absence of inoculation with *A. brasilense*. The experiment II, in the cropping season 2015/16, was carried out in a 4 x 2 factorial arrangement, with four hybrids (AS1656 Conventional, AS1656 PRO, AS1656 PRO II and AS1656 PRO III), in the presence or absence of inoculation with *A. brasilense*. There was no interaction Hybrid x Inoculation for any of the studied variables and in both experiments, i.e., was not verified specificity between hybrids and inoculation. Inoculation with *A. brasilense* did not interfere in grain yield and yield components of maize crop. Maize hybrids with similar genetic characteristics, however, with different transgenic events present difference in grain yield and yield components.

Keywords: plant growth-promoting bacteria, transgenic, *Zea mays*.

Resposta de híbridos de milho, com diferentes eventos transgênicos, à inoculação com *Azospirillum brasilense*

Resumo

A inoculação de híbridos de milho com *Azospirillum brasilense* é uma alternativa importante para aumentar a produtividade de grãos e diminuir os custos de produção dessa cultura. No entanto, existe uma especificidade entre *A. brasilense* e o híbrido a ser inoculado, principalmente híbrido de milho com evento transgênico. Assim, o objetivo do estudo foi avaliar como híbridos de milho com diferentes eventos transgênicos respondem à inoculação com *A. brasilense*. O estudo foi realizado em Cândói, Estado do Paraná, em dois experimentos, em delineamento inteiramente casualizado com quatro repetições. O experimento I, na safra 2014/15, foi realizado em arranjo fatorial 5 x 2, com cinco híbridos (AS1656 PRO II, AS1656 PRO III, AG9045 RR2, AG9045 PRO e AG9045 PRO III), na presença ou ausência de inoculação com *A. brasilense*. O experimento II, na safra 2015/16, foi realizado em arranjo fatorial 4 x 2, com quatro híbridos (AS1656 Convencional, AS1656 PRO, AS1656 PRO II e AS1656 PRO III), na presença ou ausência de inoculação com *A. brasilense*. Não houve interação Híbrido x Inoculação para nenhuma das variáveis estudadas e em ambos os experimentos, ou seja, não foi verificada especificidade entre híbridos e inoculação. A inoculação com *A. brasilense* não interferiu na produtividade de grãos e nos componentes de produção da cultura do milho. Híbridos de milho com características genéticas semelhantes, entretanto, com diferentes eventos transgênicos, apresentam diferenças nos componentes de rendimento e rendimento de grãos.

Palavras-chave: bactérias promotoras de crescimento de plantas, transgênicos, *Zea mays*.

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Respuestas de híbridos de maíz, con diferentes eventos transgénicos, a la inoculación con *Azospirillum brasilense*

Resumen

La inoculación de híbridos de maíz con *Azospirillum brasilense* es una alternativa importante para aumentar la productividad de los granos y disminuir los costos de producción del maíz. Sin embargo, existe una especificidad entre *A. brasilense* y el híbrido a ser inoculado, principalmente híbrido de maíz con evento transgénico. Así, el objetivo del estudio fue evaluar como híbridos de maíz con diferentes eventos transgénicos responden a la inoculación con *A. brasilense*. El estudio fue realizado en Cándói, Estado de Paraná, en dos experimentos, en delineamiento completamente casualizado con cuatro repeticiones. El experimento I, en la cosecha 2014/15, fue realizado en un arreglo factorial 5 x 2, con cinco híbridos (AS1656 PRO II, AS1656 PRO III, AG9045 RR2, AG9045 PRO y AG9045 PRO III), en presencia o ausencia de inoculación con *A. brasilense* de la ciudad de Buenos Aires. El experimento II, en la cosecha 2015/16, fue realizado en un arreglo factorial 4 x 2, con cuatro híbridos (AS1656 Convencional, AS1656 PRO, AS1656 PRO II y AS1656 PRO III), en presencia o ausencia de inoculación con *A. brasilense*. No hubo interacción Híbrido x Inoculación para ninguna de las variables estudiadas y en ambos experimentos, o sea, no se verificó especificidad entre híbridos e inoculación. La inoculación con *A. brasilense* no interfirió en la productividad de granos y en los componentes de producción del cultivo del maíz. Los híbridos de maíz con características genéticas similares, sin embargo, con diferentes eventos transgénicos, presentan diferencias en los componentes de rendimiento y rendimiento de granos.

Palabras clave: bacterias promotoras de crecimiento de plantas, transgénicos, *Zea mays*.

Introduction

Maize crop is part of human and animal food, being one of the main cereals produced in Brazil. According to CONAB (2018) the Brazilian production in the 2017/18 crop season was 89.2 million tons, what represents 8.8% lower than the 2016/17 crop season, mainly due to climatic events.

In order to achieve high yields levels, the maize crop needs, among other things, the nutritional balance of its plants. In this context, the nutrient most required by the crop is nitrogen, which directly influences grain yield (PORTUGAL et al., 2017). Nitrogen deficiency can affect 14-80% of maize yield (FANCELLI, 2011). This element is present in variety biochemical processes, constituting amino acids, enzymes, proteins, nucleic acids and chlorophyll, an essential molecule for photosynthesis (SANTOS et al., 2010).

Nevertheless, N is extremely complex to be managed due to a number of factors, e.g, cost of acquisition, losses and some cases negative impact on the environment. Therefore, it is of fundamental importance studies that allow the lowest economic spending (HUNGRIA et al., 2015). Thus, studies with beneficial microorganisms of soil play an important role, because they allow the economic profitability and reduce the environmental impacts. Etesami e Maheshwari (2018) showed that several

microorganisms present in the soil rhizosphere have beneficial characteristics on plant development, these microorganisms are known as Plant Growth-Promoting Bacteria (PGPB's).

The bacteria of the genus *Azospirillum brasilense* stand out among the PGPB's, mainly in the biological nitrogen fixation (BNF) (SPOLAOR et al., 2016). Bacteria of this genus when inoculated can stimulate the growth by multiple mechanisms, including the improvement in nitrogen nutrition, hormonal synthesis, and ameliorate biotic and abiotic stresses (BASHAN e DE-BASHAN, 2010), consequently increasing root growth, favoring root absorption of water and nutrients (MILLÉO e CRISTÓFOLI, 2016, MORAIS et al., 2016, MUMBACH et al., 2017).

Furthermore, it is possible to verify that one strategy used periodically to obtain high yields levels is use more productive maize hybrids. Very often, these hybrids present some transgenic event. However, some studies have shown that specificity may occur between *A. brasilense* and the maize genotype to be inoculated (MARINI et al., 2015). The relationship between *A. brasilense* and maize genotypes becomes an important factor, since the specificity between the host and the association with *A. brasilense* is one of the main factors that can generate negative influence on inoculation (MÜLLER et al., 2015).

Thus, the objective of the study was to evaluate

how hybrids of maize with different transgenic events respond to inoculation with *A. brasilense*.

Material and methods

The experiment was carried out at the Modelo farm, in Candói, Paraná State, located in the physiographic region known with Terceiro Planalto Paranaense, with geographic coordinates of 25° 30'10" South latitude and 51° 48' 26" West longitude and altitude is approaching 1,100 m. According to Köppen, the climate is classified as Humid Subtropical (Cfb) (MAAK, 1968).

The experiment was carried out in two cropping season, 2014/15 and 2015/16. Climatic data for these periods were acquired at the Meteorological Station of the State University of Midwest (UNICENTRO), CEDETEG Campus (Figure 1). The soil of the experimental area is classified as Brown Latosol (EMBRAPA, 2013).

The experiment I, in the cropping season 2014/15, was carried out in a 5x2 factorial arrangement, with five hybrids (AS1656 PRO II, AS1656 PRO III, AG9045 PRO, AG9045 PRO III and AG9045 RR2), in the presence or absence of inoculation with *A. brasilense*. The experiment II, in the cropping season 2015/16,

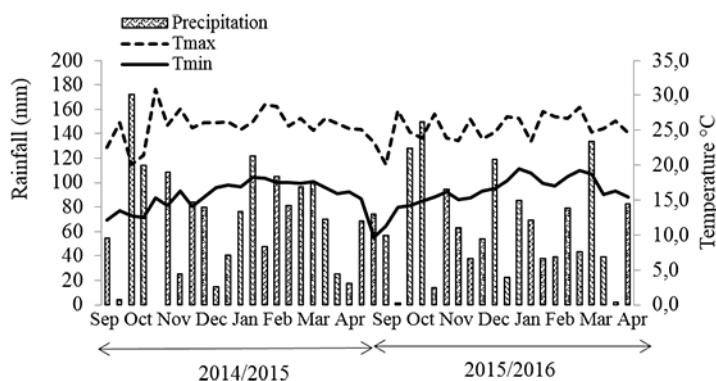


Figure 1. Rainfall, maximum temperature and minimum temperature for each month during the period of experiment conduction in the cropping season 2014/15 and 2015/16.

was carried out in a 4 x 2 factorial arrangement, with four hybrids (AS1656 Conventional, AS1656 Pro, AS1656 Pro II and AS1656 Pro III), in the presence or absence of inoculation with *A. brasilense*. The studies were carried out in a completely randomized design with four replications. Others information about the experiments are shown in Table 1.

Inoculant was applied in the seed, manually, in the shadow and two hours before start sowing. The inoculant used was AzoTotal™, which is a liquid formulation containing strains Ab-V5 (CNPSO 2083) and Ab-V6 (CNPSO 2084) of *A. brasilense* at the concentration of 2 x 10⁸ CFU mL⁻¹, with a recommended dose of 100 mL 60,000 seeds⁻¹.

In both cropping season, the experiments were carried out in a no-tillage system, in succession with black oats (*Avena strigosa* L.). Desiccation was performed with glyphosate herbicide (720 g ha⁻¹ a.i.), 30 days before sowing. The experimental units

were composed of seven rows, the rows were 50 cm spaced apart and length of 6 m. The useful area was composed for the three central lines. The 0.50 m of each end was not considered for evaluations.

The variables studied were: yield, thousand-grain mass (TGM), number of rows per ear, number of grains per row, number of grains per ear, plant height, height of insertion of first ear and damaged kernels.

The harvest was carried out when all plants reached the physiological maturation stage. Firstly, ten ears in sequence were harvested in the third row in the useful experimental area, with these ears was determined the number of grains per row, number of grains per ear. After that, yield was estimated by the harvest of the remaining ears of the three central lines and afterwards the weighing and correction of moisture to 14%, the value obtained was converted into kg ha⁻¹. A sample was taken and used to determine the thousand-grain mass by counting and weighing 300 grains.

Table 1. Management of the experimental area for both cropping season. Guarapuava, PR-2018.

Activity	Experiment/Cropping season	
	Experiment I 2014/15	Experiment II 2015/16
Sowing	09/14/2014	09/24/2015
Spacing (m)	0.45	0.42
Population (plants ha ⁻¹)	75.000	85.000
<i>Basal fertilization (kg ha⁻¹)</i>		
N	49.4 kg ha ⁻¹	49.4 kg ha ⁻¹
P ₂ O ₅	133 kg ha ⁻¹	133 kg ha ⁻¹
K ₂ O	38 kg ha ⁻¹	38 kg ha ⁻¹
<i>Nitrogen top-dressing</i>		
V3 (100 ou 75 kg ha ⁻¹)	10/11/2014	10/16/2015
V5 (100 ou 75 kg ha ⁻¹)	10/24/2014	10/28/2015

The height of insertion of the first ear and height of plant was measured with a graduated ruler from the ground up to the insertion of first ear and up to the insertion the flag leaf, respectively. Were measured three

plants of the third central line of each sampling unit.

The data were submitted to analysis of variance and afterwards the means were compared by the Tukey test (for hybrids) and by *t* test (for presence or absence of inoculation) at 5% probability. Were performed analyzes using the SISVAR 5.6 software.

Results and discussion

According to Table 2, the source of variation hybrid showed significant difference for all variables analyzed and for both experiments. Furthermore, the source of variation inoculation, in experiment I and II, did not present significance for the variables analyzed. There was not interaction between hybrid and Inoculation for all variable analyzed in the experiment I. For experiment II, was observed significant interaction between hybrid and *A. brasilense* only for grains per row.

The difference in the conditions of these studies makes it interesting to discuss them separately. Thus, it will be easier to understand the differences between maize hybrids evaluated in presence or absence of inoculation with *A. brasiliense*.

Table 2. Mean square values from analysis of variance of the effects of presence and absence of inoculation with *A. brasiliense* in maize hybrids with different transgenic events. Guarapuava, PR, Brazil, 2018.

Source of variation	DF	Mean square values							
		Yield (kg ha ⁻¹)	TGM (g)	Damaged grains (%)	Grain per row	Row	Grain per ear	Plant height (cm)	Height of insertion of first ear (cm)
Experiment I (2014/2015)									
Block	3	417995.40 ns	185.97 ns	0.89 ns	1.92 ns	0.23 ns	867.87 ns	46.76 ns	31.23 ns
Hybrid (H)	4	15322001.22 **	482.60 **	1.87 ns	4.63**	11.76 **	12070.19 **	378.21 **	473.22 **
Inoculation (I)	1	602702.50 ns	30.28 ns	1.59 ns	0.65 ns	0.20 ns	595.21 ns	1.22 ns	6.40 ns
H X I	4	34230.00 ns	11.66 ns	1.03 ns	1.98 ns	0.39 ns	154.57 ns	62.41 ns	10.28 ns
Error	27	508206.40	111.69	0.79	2.04	0.32	993.58	65.52	97.17
Mean	-	14747	417.60	1.60	30.85	15.62	482.06	264.02	148.05
CV %	-	4.83	2.53	55.32	4.63	3.64	6.54	3.07	6.66
Experiment II (2015/2016)									
Block	3	1579534.7 *	80.28 ns	0.5545 ns	3.7745 ns	0.1211 ns	1368.001 ns	29.2083 ns	25.2812 ns
Hybrid (H)	3	22346662.54 **	6046.16 **	1.9675 *	16.672**	0.9661*	8692.537**	58.5416*	129.3645*
Inoculation (I)	1	499500.12 ns	189.68 ns	0.5304 ns	5.78 ns	0.0253 ns	1914.257 ns	10.125 ns	30.1312 ns
H X I	3	103891.87 ns	15.88 ns	0.7016 ns	11.7991*	0.1019 ns	2426.527 ns	6.875 ns	22.2812 ns
Error	21	353336.16	127.57	0.5815	3.11	0.2163	1181.493	18.5178	28.5669
Mean	-	10821.81	430.43	1.281	27.63	16.34	451.909	234.687	128.093
CV %	-	5.49	2.62	59.49	6.38	2.85	7.61	1.83	4.17

TGM - thousand-grain mass; CV = Coefficient of variation; * Significant to 5%; ** Significant to 1% by F test; ns - not significant.

Experiment I (2014/15)

In the experiment I, when observing the interaction, hybrids and Inoculation, it was verified that there is no statistical difference for grain yield. However, when the means are observed separately, was observed that there is difference between maize hybrids for grain yield (Table 3).

According to Table 3, for all the hybrids studied, no statistical differences in yield were observed between presence and absence of inoculation. The results found in our study differ from those found by Brum et al. (2016) in which inoculation with *A. brasilense* showed a positive effect on maize crop yield in two different cropping season 2012/13 and 2013/14.

In the presence or absence of inoculation, it was found that all the hybrids evaluated were statistically different from the hybrid AG 9045 RR2. The maximum yield, in both situations, was verified with the hybrid AS 1656 PRO III. It was also verified that the hybrid AG 9045 RR2 presented the lowest grain yield and was

statistically different from AG 9045 PRO and AG 9045 PRO III, that presented different transgenic events.

The results obtained in our study differ from those obtained by Bartchechen et al. (2010), according these authors, when studying the interaction between genotypes and environment, some hybrids respond differently when inoculated with *A. brasilense*. Brusamarello-Santos et al. (2017), studied the efficiency of colonization of *A. brasilense* in two different maize hybrids, the authors verified that there is a difference in the metabolic approach between plant and bacteria. Lana et al. (2012), also analyzing the effects of inoculation with *A. brasilense* on maize hybrids grown in cropping season, local and with different management, observed that inoculation provided an increase of approximately 15% and 7.4% in grain yield in the first and second cropping season, respectively.

For thousand-grain mass, there was no difference for any of the hybrids studied, either in the presence or in absence of inoculation.

Table 3. Yield (kg ha⁻¹) and thousand-grain mass (TGM - g) of different maize hybrids, in the presence or absence of inoculation with *A. brasilense* in the cropping season 2014/15. Guarapuava, PR, 2018.

Hybrids	Yield (kg ha ⁻¹)			TGM (g)		
	Presence of Inoculation	Absence of Inoculation	Mean	Presence of Inoculation	Absence of Inoculation	Mean
AS 1656 PRO III	16406 a	15981 a	16194	426.54 a	423.47 a	425.01
AS 1656 PRO II	15868 ab	15610 ab	15739	404.84 a	404.10 a	404.47
AG 9045 PRO	14766 b	14619 ab	14692	417.92 a	421.29 a	419.61
AG 9045 PRO III	14535 b	14442 b	14489	420.11 a	421.30 a	420.71
AG 9045 RR2	12774 c	12471 c	12623	418.61 a	417.86 a	418.24
Mean	14870	14625	14747	417.604	417.604	417.60

Means followed by lowercase letters in the column and upper case in the row do not differ, among them by Tukey's test and T test, respectively, at 5%.

In Table 4, it was verified that there was difference of grain yield between the hybrids evaluated. The findings of our study allow us

to infer that maize hybrids with similar genetic characteristics, however, with different transgenic events present difference in grain yield, e.g, the

Table 4. Yield, thousand-grain mass (TGM), damaged grain, insertion height of first ear, plant height, number of row per ear, number of grain per row and number of grain per ear, of different maize hybrids, in average of presence and absence of inoculation with *A. brasilense* in the cropping season 2014/15. Guarapuava, PR, 2018.

Hybrid	Yield (kg ha ⁻¹)	TGM (g)	Damaged grain (%)	Insertion height (cm)	Plant height (cm)	Row	grain per row	grain per ear
AS 1656 PRO III	16194 a	425.00 a	1.86 a	159 a	274 a	16.6 a	30.2 a	503.1 ab
AS 1656 PRO II	15739 a	404.47 b	1.84 a	152 ab	267 ab	17.2 a	31.3 a	538.1 a
AG 9045 PRO	14692 b	419.61 ab	0.94 a	142 b	260 b	14.8 b	32.0 a	473.5 bc
AG9045PROIII	14488 b	420.71 a	1.26 a	146 ab	263 ab	14.9 b	30.4 a	451.7 c
AG 9045 RR2	12623 c	418.24 ab	2.13 a	141 b	256 b	14.6 b	30.4 a	443.9 c
Mean	14747.2	417.606	1.606	148	264	15.62	30.86	482.06

Means followed by same letters do not differ among them by Tukey's test, at 5%.

hybrid AG 9045 RR2 presented lower and statistically different grain yield in comparison with its isogenic, AG 9045 PRO and AG 9045 PRO III.

It was verified that the hybrid AS 1656 PRO III, that presented the higher yield, provided higher height of ear insertion and height of plant, more number of

rows per ear and more number of grains per ear in comparison with AG 9045 RR2, that present the lower yield. There was no difference in thousand-grain mass between these hybrids. Differences between maize hybrids was also reported by Torres et al. (2013).

Table 6. Yield, thousand-grain mass (TGM), damaged grain, insertion height of first ear, plant height, number of row per ear, number of grain per row and number of grain per ear, of different maize hybrids, in average of presence and absence of inoculation with *A. brasilense* in the cropping season 2015/16. Guarapuava, PR, 2018.

Hybrid	Yield (kg ha ⁻¹)	TGM (g)	Damaged grain (%)	Insertion height (cm)	Plant height (cm)	Row	grain per row	grain per ear
AS 1656 PRO	12613 a	398.35 d	1.99 a	134 a	237 a	16.65 a	29.53 a	492.5 a
AS 1656 PRO II	11650 b	419.01 c	0.86 b	126 b	233 a	16.62 a	27.95 ab	464.6 ab
AS 1656 Conv.	10240 c	443.22 b	1.21ab	126 b	232 a	16.11 ab	26.66 b	429.5 b
AS1656PROIII	8785 d	461.16 a	1.07 ab	126 b	237 a	15.97 b	26.37 b	421.1 b
Mean	10822	430.435	1.28	128	234.75	16.35	27.65	451.925

Means followed by same letters do not differ among them by Tukey's test, at 5%.

Experiment II (2015/16)

Similar to the observed in experiment I (Table 3), it was observed in the Table 5, that the same hybrids, but with different transgenic events, presented statistic difference in grain yield. The lowest yield was found in the hybrid AS1656 PRO III, which differed statistically from the others. Our results reinforce that different transgenic events in similar hybrids might provide differences in grain yield of maize crop.

In contrast, the highest values for TGM were verified in the hybrids that had the lowest yields

(AS1656 Conventional and AS1656 PRO III). The results of this study allow us to infer that maize crop yield comes from the addition of several yield components, not just TGM. Grain yield is a very complex character, which is the result of different yield components, i.e, so this variable is affected by almost all components of crop yield (SOUZA et al., 2014).

The results obtained in our study indicate that there was no influence of inoculation in yield and TGM of maize crop. Controversial results was obtained by Müller et al., (2015); Portugal et al., (2016); Galindo et al. (2017) these authors found

Table 5. Yield (kg ha⁻¹) and thousand-grain mass (TGM - g) of different maize hybrids, in the presence or absence of inoculation with *A. brasilense* in the cropping season 2015/16. Guarapuava, PR, 2018.

Hybrids	Yield (kg ha ⁻¹)			TGM (g)		
	Presence of Inoculation	Absence of Inoculation	Mean	Presence of Inoculation	Absence of Inoculation	Mean
AS1656 PRO	12720 A a	12505 A a	126125	400 A b	396 A b	398
AS1656 PRO II	11767 A a	11531 A a	11649	421 A b	416 A b	418.5
AS1656 Conv.	10515 A b	9964 A b	10239	462 A a	439 A a	450.5
AS1656 PRO III	8783 A c	8786 A c	8784	447 A a	460 A a	453.5
Mean	10946	10696	108213	432.5	427.75	430.12

Means followed by lowercase letters in the column and upper case in the row do not differ, among them by Tukey's test and T test, respectively, at 5%.

positives responses in yield and yield components of maize crop when inoculated with *A. brasilense*. The contribution of *A. brasilense* can make plant more

efficient in the absorption of water and nutrients, consequently may contribute to increase yield (HUNGRIA et al., 2015) and can promote plant

growth through the production of phytohormones and siderophores (HUZAR-NOVAKOWISKI et al., 2011). Similarly of our findings, Vogt et al., (2014) and Sangoi et al., (2015) did not find positive results with inoculation. Further studies are required to investigate the effect of inoculation with *A. brasilense* in maize hybrids with different transgenic events.

According to Table 6, there was difference in grain yield between the hybrids studied in the 2015/16 cropping season. The highest yield was obtained with the hybrid 1656 PRO, which differed from all the others evaluated. We can observe that the hybrid AS1656 PRO, presented higher height of insertion of the first ear, number of rows, grains per rows and grains per ear when compared to hybrid AS 1656 PRO III, that

presented the lower yield. Pias et al., (2018), previously reported differences between maize hybrids.

Our findings highlight that maize hybrids with genetic similarities, but with different transgenic events, might present statistical difference in grain yield. Further studies are required to better understand the relation between maize hybrids with transgenic and inoculation with *A. brasilense*.

Conclusion

Inoculation with *A. brasilense* did not interfere in grain yield and yield components of maize crop. Our results indicate that maize hybrids with similar genetic characteristics, however, with different transgenic events present difference in grain yield.

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