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CHARACTERIZATION OF *Colletotrichum lindemuthianum* ISOLATES IN COMMON BEAN FROM PARANA STATE, BRAZIL

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ABSTRACT - Anthracnose is one of the most important diseases of common bean caused by *Colletotrichum lindemuthianum* fungus. The study objectified to characterize isolates collected in crops from Parana State. Thirty-four isolates of *C. lindemuthianum* were tested on a set of 12 international bean differential cultivars for anthracnose in *Phaseolus vulgaris* L. Disease reactions were rated visually using a severity scale from 1 to 9.Twenty-five races were identified: 2, 3, 10, 11, 15, 27, 31, 63, 64, 73, 75, 79, 81, 82, 83, 90, 91, 93, 95, 259, 283, 287, 339, 346 and 351. This was the first report of races 82, 90, 259, 283, 287, 346 and 351 in Parana State, with the first report of race 3, 15 and 63 in Brazil. Race 3 presented the highest frequency of occurrence (14.7%), followed by races 2, 64, 91, 95 and 351 (5.9%). Races 64 and 73 presented compatibility reactions only with Mesoamerican cultivars. Races 3, 10, 27, 31, 75, 79, 81, 82, 91, 93, 259, 283, 287, 346 and 351 showed compatibility reactions with both Andean and Mesoamerican cultivars. All Andean cultivars presented compatibility reaction with isolates being incompatible with PI 207262, TU, AB 136 and G 2333 cultivars.

KEY WORDS: *Phaseolus vulgaris* L., anthracnose, pathogen variability, races monitoring.

CARACTERIZAÇÃO DE ISOLADOS DE *COLLETOTRICHUM LINDEMUTHIANUM* EM FEIJÃO COMUM NO ESTADO DO PARANÁ, BRAZIL

RESUMO – Antracnose é uma das mais importantes doenças do feijão comum causada pelo fungo *Colletotrichum lindemuthianum.* O estudo objetivou caracterizar isolados coletados em lavouras comerciais do estado do Paraná. Trinta e quatro isolados de *C. lindemuthianum* foram testados em um conjunto internacional de 12 cultivares diferenciadoras para reação à antracnose em *Phaseolus vulgaris* L. Os sintomas da doença foram classificados visualmente usando uma escala de severidade de 1 a 9. Vinte e cinco raças foram identificadas: 2, 3, 10, 11, 15, 27, 31, 63, 64, 73, 75, 79, 81, 82, 83, 90, 91, 93, 95, 259, 283, 287, 339, 346 e 351. Este foi o primeiro relato das raças 82, 90, 259, 283, 287, 346 e 351 no estado do Paraná, com o primeiro relato das raças 3, 15 e 63 no Brasil. A raça 3 apresentou a frequência de ocorrência mais elevada (14,7%), seguida pelas raças 2, 64, 91, 95 e 351 (5.9%). As raças 64 e 73 mostraram reações de compatibilidade somente com cultivares Mesoamericanas. As raças 3, 10, 27, 31, 75, 79, 81, 82, 91, 93, 259, 283, 287, 346 e 351 apresentaram reações de compatibilidade com ambas cultivares Andinas e Mesoamericanas. Todas as cultivares Andinas apresentaram reações de compatibilidade com os isolados sendo incompatíveis com as cultivares diferenciadoras PI 207262, TU, AB 136 e G 2333.

PALAVRAS-CHAVE: *Phaseolus vulgaris* L., antracnose, variabilidade patogênica, monitoramento de raças.

INTRODUCTION

Phaseolus vulgaris L. is a crop with economic, social and nutrition importance, especially in developing Countries of Latin America, East and Southern Africa (Broughton et al., 2003). Mean world production of common bean was around 23.300.000 tons (FAO, 2016). Brazil has the third highest production (2,600,000 tons). Parana State stands out as the main Brazilian State in production, with 23% of total produced (Conab, 2016).

However, common bean cultivation may be affected by the occurrence of phytosanitary problems, with diseases responsible for low yield and depreciation in quality of final product. One of these, anthracnose, is caused by *Colletotrichum lindemuthianum* (Sacc.) Scrib fungus, causing losses of up to 100%, when using susceptible genotypes, infected seed and when climatic conditions are favorable to the pathogen development (Pastor-Corrales and Tu, 1989; Carbonell et al., 1999; Bianchini et al., 2005; Mendéz-Vigo et al., 2005).

Several authors reported a significant number of distinct races identified. Nunes et al. (2013), studied the incidence of *C. lindemuthianum* fungus in *P. vulgaris* and found that there was the reported of approximately 247 races identified in 28 countries. One of the most efficient measures for controlling *C. lindemuthianum* has been the use of genetic resistance, since there are reductions in productions costs, besides diminution damage to the environment, reducing, or even avoiding pesticides use (Mahuku and Riascos, 2004; Costa and Rava, 2009). However, this use is difficult because of the occurrence of high variability of by this pathogen races (Rava et al., 1994; Ishikawa et al., 2005).

In Brazil, 73 races of *C. lindemuthianum* have already been identified, being Parana State, standing out due to the greatest variability, with 44 races, accounting for 60.3% of Brazilian races. Wide distribution of the crop in Parana and favorable climatic conditions, allows to the fungus presents its host available on a large scale and in all cultivation seasons (water, drought and winter), what may favors its spread in this region (Nunes et al., 2013). Thus, the present study aimed to characterize isolates of *Colletotrichum lindemuthianum* collected in common bean cultivations, in several counties of Parana State.

MATERIAL AND METHODS

The experiments were conducted under greenhouse conditions and at Laboratory of Common Bean Crop Breeding and Molecular Biology belonging to Research Center Applied to Agriculture, State University of Maringa, Parana State, Brazil.

Samples of seeds, leaves, stems and pods with anthracnose symptoms were collected from common bean cultivations in the period between July, 2013 and November, 2014, in Counties of Parana State (Guarapuava, Irati, Maringa and Prudentopolis) with repetitions of the same local collections, but in different years, exception for Cascavel (only in 2014 year). Samples were correctly identified and placed in airtight bags, preventing deterioration. Subsequently, samples were taken to laboratory, in order to characterize *Colletotrichum lindemuthianum* races. Thirty four isolates were obtained from typical lesions with symptoms.

The isolates were named as: CL01, CL02, CL03, CL04, CL05 and CL06 (Tangara cultivar); CL07 and CL08 (Carioca group, without genotype identification); CL09, CL10 and CL12 (BRS Campeiro); CL 11 (Black group); CL13, CL14 and CL15 (Black group) and CL 16 (Carioca group); CL17 (Crioulo group), CL18, CL22 and CL28 (Juriti cultivar), CL19, CL24 and CL25 (LEC - 01 10 access), CL20 (Crioulo group), CL21 and CL23 (Perola Cultivar), CL26 and CL27 (LP 145 genotype) and CL29 (Treatment 19 from an experimental planning - T19). CL30, CL31, CL32 and CL33 isolates (Prudentopolis County) had origin from plants lesions in Carioca group genotype and CL34 from BRS Esplendor cultivar.

C. lindemuthianum monosporic cultures were put in glass tubes with potato – dextrose - agar medium and subsequently transferred to those containing common bean pods immersed in agar agar medium according to the methodology proposed by Mathur et al. (1950). Glass tubes were kept in BOD conditions (20º± 2º C for 15 days) obtaining spores.

Following incubation period, pods were transferred to Becker with water distilled and sterilized, in order to generate a suspension that was passed by double layer gauze creating spores suspension. Each one was counted seven times with the aid of a Neubauer-Preciss chamber, adjusting for 1.2 x 10^6 spore's mL^{-1} concentration in dilutions.

C. lindemuthianum differentiation races were done by using 12 cultivars designated by a binary number: Michelite: 1; Michigan Dark Red Kidney: 2; Perry Marrow: 4; Cornell 49-242: 8; Widusa: 16; Kaboon: 32; Mexico 222: 64; PI 207262: 128; TO: 256; TU: 512; AB 136: 1024 and G 2333: 2048. The result of the sum of each number corresponding to each cultivar was the race designation.

Each one of 12 cultivars was sown in plastic trays containing soil and sterilized organic material, maintained in a plastic green house in order to develop primary leaf stage. Fifteen seedlings were inoculated in both sides of the leaves, in each differential cultivar with the aid of air compression De Vilbiss Model. Trays were kept in a mixture chamber for approximately 72 hours at 20º± 2o C, alternating 12 hours of light and 12 hours of dark with

100% relative humidity. Eight days after inoculating plants received scores as suggested by Pastor-Corrales (1991), from 1 to 9. Plants scored 1 to 3 were considered resistant while those ones scored 4 to 9 were considered susceptible.

RESULTS AND DISCUSSION

The 34 isolates of *C. lindemuthianum* evaluated in this study showed different patterns of virulence and allowed the identification of 25 physiological races: 2, 3, 10, 11, 15, 27, 31, 63, 64, 73, 75, 79, 81, 82, 83, 90, 91, 93, 95, 259, 283, 287, 339, 346, 351 (Table 1).

Race 3 showed the highest occurrence frequency (14.7%), corroborating with results published in literature. This race presents significant geographical distribution in the world, having been established in Countries such as South Africa, Argentina, Bulgaria, Colombia, Ecuador, Spain, United States, India, Mexico, Peru and Dominican Republic (Pastor-Corrales et al., 1995; Balardin et al., 1997; Falconi et al., 2003; Ansari et al., 2004; Mahuku and Riascos, 2004; Sharma et al., 2007; Ferreira et al., 2008; Muth and Liebenberg, 2009; Genchev et al., 2010; Padder et al., 2010; Mohammed, 2013).

However, this is the first report of this race occurrence in Brazil. Race 3 was characterized from five isolates: three collected in Cascavel County and two collected in Maringa County, both in Parana State.

Races 15, 63 and 339 have also been identified by the first time in Parana State. However, races 75 and 339 have already been related in Santa Catarina and Mato Grosso do Sul, respectively (Alzate-Marin and Sartorato, 2004). Races 15 and 63 showed low frequency, but race 15 has already been found in Colombia and Ecuador Countries (Pastor-Corrales et al., 1995; Balardin and Kelly, 1998; Mahuku and Riascos, 2004; Genchev et al., 2010), while race 63 was reported only in Argentina (Sicard et al., 1997). In Brazil, this is the first occurrence relate of this race. Races 2, 64, 91, 95 and 351 presented occurrence frequency of 5.9%.

Race 2, only compatible with Andean differential cultivar Michigan Dark Red Kidney was identified in Cascavel County (one isolate). It is characterized by being a race with broad geographic distribution and its occurrence has been reported in Argentina, Brazil, Bulgaria, Ecuador, Greece, Mexico, Peru, Dominican Republic, Kenya, Tanzania and Uganda (Pastor-Corrales et al., 1995; Balardin et al., 1997; Sicard et al., 1997; Ombiri et al., 2002; Mahuku and Riascos, 2004; Gonzales-Chavira et al., 2004; Ansari et al., 2004; Sharma et al., 2007; Bardas et al., 2007; Genchev et al., 2010; Padder et al., 2010; Nunes et al., 2013).

Race 64 was characterized from two isolates from Maringa and Irati. It is one of the most widespread and incident race in common bean producing regions, corroborating the results obtained by Ishikawa et al. (2005); Sansigolo et al. (2008); Felipin-Azevedo et al. (2014).

Races 64 and 73 showed compatibility reaction only with Mesoamerican cultivars. Nonetheless, races 3, 10, 11, 15, 27, 31, 63, 75, 79, 81, 82, 83, 90, 91, 93, 95, 259, 283, 287, 339, 346 and 351 presented compatibility reaction with both cultivars origin Andean and Mesoamerican. All Andean differential cultivars revealed compatibility reaction with the isolates, excepting for Kaboon cultivar, incompatible with all isolates and compatible only with race 63. All isolates were incompatible with PI 207262, TU, AB 136 and G 2333 cultivars, becoming important resistance sources for use in common bean improvement programs, aiming anthracnose control in Parana State. Mesoamerican cultivar TO is considered an important resistance source. However, its resistance was overcome by 259, 283, 287, 339, 346 and 351 races. Isolates were from Cascavel, Irati and Prudentopolis, one isolate in each County, respectively (Table 1).

Races 2, 10, 27, 31, 64, 73, 75, 79, 81, 83, 91, 93 and 339 had been described in Brazil, mostly in Parana State, that proved to be the main State with the greatest genetic variability of the pathogen, as pointed out the literature review conducted by Nunes et al. (2013). Races 82, 90, 259, 283, 287, 346 and 351 of *C. lindemuthianum* are being described for the first time on the world scenario, demonstrating pathogen genetic variability.

Race 73 was characterized from material collected in Guarapuava County (one isolate). This race is characterized as one with the highest incidence in the world, being widely disseminated in common bean producing countries of North, Central and South America (Balardin et al., 1997). In Brazil, it also occurs frequently (Rava et al., 1994; Balardin et al., 1997; Carbonell et al., 1999; Gonçalves-Vidigal et al., 2008a) and in Parana State stands out as one of most incidents (Carneiro, 1999; Rava et al., 1994; Thomazella et al., 2002).

Race 75 presented frequency around 3%, having been found in Maringa (one isolate). This race is not very widespread in the world, but it was found in Brazil, one of the countries with the largest fungus pathogenic variability, in Espirito Santo, Parana and Santa Catarina States (Rava et al., 1994; Thomazella et al., 2002; Gonçalves-Vidigal et al., 2008b). Its first occurrence in Parana State was in 2008, as reported by Sansigolo et al. (2008).

Table 1 – Identification of physiological races from 34 *C. lindemuthianum* isolates collected in Parana State according to differential cultivars set reactions (Maringa/PR, 2015).

Isolate	County	Differential cultivars set												Races
		A	B	C	D	E	F	G	H	I	J	K	L	
CL01	Cascavel	-	+	-	-	-	-	-	-	-	-	-	-	2
CL02	Cascavel	+	+	-	-	-	-	-	-	-	-	-	-	3
CL03	Cascavel	+	+	-	-	-	-	-	-	-	-	-	-	3
CL04	Cascavel	+	+	-	-	-	-	-	-	-	-	-	-	3
CL05	Cascavel	+	+	-	+	+	-	-	-	-	-	-	-	27
CL06	Cascavel	+	+	-	+	+	-	-	-	+	-	-	-	283
CL07	Guarapuava	-	+	-	+	-	-	-	-	-	-	-	-	10
CL08	Guarapuava	+	+	+	+	-	-	-	-	-	-	-	-	15
CL09	Guarapuava	+	-	-	+	-	-	+	-	-	-	-	-	73
CL10	Guarapuava	+	-	-	-	+	-	+	-	-	-	-	-	81
CL11	Guarapuava	-	+	-	-	+	-	+	-	-	-	-	-	82
CL12	Guarapuava	+	-	+	+	+	-	+	-	-	-	-	-	93
CL13	Irati	+	+	+	+	+	+	-	-	-	-	-	-	63
CL14	Irati	-	-	-	-	-	-	+	-	-	-	-	-	64
CL15	Irati	+	+	-	-	+	-	+	-	-	-	-	-	83
CL16	Irati	+	+	+	+	+	-	+	-	+	-	-	-	351
CL17	Maringa	-	+	-	-	-	-	-	-	-	-	-	-	2
CL18	Maringa	+	+	-	-	-	-	-	-	-	-	-	-	3
CL19	Maringa	+	+	-	-	-	-	-	-	-	-	-	-	3
CL20	Maringa	-	-	-	-	-	-	+	-	-	-	-	-	64
CL21	Maringa	+	+	-	+	-	-	+	-	-	-	-	-	75
CL22	Maringa	+	+	+	+	-	-	+	-	-	-	-	-	79
CL23	Maringa	-	+	-	+	+	-	+	-	-	-	-	-	90
CL24	Maringa	+	+	-	+	+	-	+	-	-	-	-	-	91
CL25	Maringa	+	+	+	+	+	-	+	-	-	-	-	-	95
CL26	Maringa	+	+	-	-	-	-	-	-	+	-	-	-	259
CL27	Maringa	+	+	+	+	+	-	-	-	+	-	-	-	287
CL28	Maringa	+	+	-	-	+	-	+	-	+	-	-	-	339
CL29	Maringa	+	+	+	+	+	-	+	-	+	-	-	-	351
CL30	Prudentopolis	+	+	-	+	-	-	-	-	-	-	-	-	11
CL31	Prudentopolis	+	+	+	+	+	-	-	-	-	-	-	-	31
CL32	Prudentopolis	+	+	-	+	+	-	+	-	-	-	-	-	91
CL33	Prudentopolis	+	+	+	+	+	-	+	-	-	-	-	-	95
CL34	Prudentopolis	-	+	-	+	+	-	+	-	+	-	-	-	346

*Differential cultivars used for *C. lindemuthianum* characterization races, followed by its respective binary values (Pastor-Corrales, 1991): A- Michelite (1); B- Michigan Dark Red Kidney (2); C- Perry Marrow (4); D- Cornell 49-242 (8); E- Widusa (16); F- Kaboon (32); G- Mexico 222 (64); H- PI 207262 (128); I- TO (256); J- TU (512); K- AB 136 (1024); L- G2333 (2048).

Races 10, 11, 15, 27, 31, 63, 79, 81, 82, 83, 90, 93, 259, 283, 287, 339 and 346 presented lower occurrence frequency (17 isolates). Races 10, 15, 81, 82 and 93 were found

in Guarapuava (five isolates). Other races were found in: Prudentopolis (11, 31 and 346 races - three isolates), Cascavel (27 and 283 races - two isolates), Irati (63 and 83 races-two isolates) and Maringa (79, 90, 259, 287 and 339 races -five isolates).

Races 10, 11, 27, 81 and 83 have been reported in Brazil, in Parana State (Mahuku and Riascos, 2004; Sansigolo et al., 2008). Race 79 race was reported by Mesquita et al. (1998), with occurrence reports in Espirito Santo State. Race 83 was also observed in this State (Rava et al., 1994), Santa Catarina (Thomazella et al., 2002; Gonçalves-Vidigal et al., 2008a), Minas Gerais (Talamini et al., 2004) and Parana (Sansigolo et al., 2008). Race 31 race has been reported only in Brazil, in common bean producing regions in Rio Grande do Sul (Balardin et al., 1997), São Paulo (Carbonell et al., 1999) and Parana (Thomazella et al., 2002).

Race 81 was found in Guarapuava and has wide geographical spread in Argentina, South Africa, Brazil, Bulgaria, China and Japan (Rava et al., 1994; Balardin et al., 1997; Balardin and Kelly, 1998; Thomazella et al., 2000; Thomazella et al., 2002; Mahuku and Riascos, 2004; Talamini et al.,2004; Alzate-Marin and Sartorato, 2004; Bonett et al., 2008; Gonçalves-Vidigal et al., 2008a; Sansigolo et al., 2008; Wang et al., 2008; Muth and Liebenberg, 2009; Gonçalves-Vidigal et al., 2009; Genchev et al., 2010; Pinto et al., 2010; Mohammed, 2013).

In Brazil, this race has been reported in Bahia (Rava et al., 1994), Pernambuco (Alzate-Marin et al., 1999), São Paulo (Carbonell et al., 1999), Minas Gerais (Talamini et al., 2004), Parana (Sansigolo et al., 2008) and Santa Catarina (Gonçalves-Vidigal et al., 2008a) States. It is one of the races with higher occurrence in Parana, widely disseminated as one of the most frequent. Races 91 and 95 races were found in Maringa (two isolates) and Prudentopolis (two isolates). With one isolate of each race, there was an occurrence frequency around 3%. Race 95 was reported by Balardin et al. (1997) in Rio Grande do Sul and by Gonçalves-Vidigal et al. (2008a) in a study of races identification in Santa Catarina State.

PI 207262, TU, AB136 and G2333 Mesoamerican differential cultivars showed resistance reaction to all physiological races. Michelite Mesoamerican cultivar and Michigan Dark Red Kidney Andean cultivar proved susceptible to most races. TO Mesoamerican differential cultivar, characterized as one of the main resistance sources showed susceptibility pattern to races 259, 283, 287, 339, 346 and 351. Kaboon Andean cultivar, possessing *Co-1*^{2} allele, had its resistance overcome only by race 63, being incompatible with all other isolated, characterized as one of the main sources of resistance to anthracnose (Table 1).

This study revealed that races 73, 75 and 81, considered the most frequent in Parana State, still exhibit the same virulence pattern in common bean producing regions in this State, where collections were conducted (Rava et al., 1994; Carneiro, 1999). According to Talamini et al. (2004), the predominance of race 81 over the years in common bean producing regions, in Brazilian States demonstrates its wide adaptation to different regions, facilitated by free trade in grain used as seeds between States corroborating with previous results.

Results obtained demonstrate the importance of conducting periodic surveys to monitor the races physiological variability within each cultivation region, since each one presents its peculiarities about adopted management, environmental conditions and cultivars preference. Studies conducted by Talamini et al. (2004), *C. lindemuthianum* spread to new locations is also favored by pathogen inoculums potential among agricultural years that tends to increase, by the fact that producers reuse their grain as seeds for crops in the same area.

According to obtained results, it is observed that race 3 showed the highest occurrence frequency among the Counties where there was isolated collection. Pathogen diversity is quite variable in South America, contrasting with results obtained by Balardin et al. (1997), demonstrating that *C. lindemuthianum* races are more variable in Central America than South America or North America. Similarly, there was contrast of obtained results with the work developed by Pastor-Corrales (1996) which reported that *C. lindemuthianum* population in Central America was more diversified than in Andean regions.

C. lindemuthianum frequencies race in several regions, where common bean crop is affected by the pathogen in the world varies. When comparing Brazilian races more frequently with other Countries, such as Nicaragua, Mexico and United States it is possible to verify a significant difference among the frequencies of identified races. In these Countries, it is common to find more virulent races (264, 320, 1545 and 1608) (González et al., 1998). However, in Brazil, there is a predominance of less virulent races (Thomazella et al., 2002).

Compatibility reaction in the set of differential cultivars revealed that Andean cultivar Michigan Dark Red Kidney was the most susceptible to all isolates showing, phenotypic patterns of 85%, followed by Michelite (76%), Cornell 49-242 (58%), Widusa (52%), Mexico 222 (52%), Perry Marrow (29%), TO (17.6%) and Kaboon (2.9%). PI 207262, TU, AB 136 and G2333 differential cultivars with 100% of resistance, being incompatible to all isolates. Resistance of PI 207262, TU, AB 136 and G2333 Mesoamerican cultivars corroborate results reviewed by Nunes et al. (2013), different from TO cultivar, that possess *Co-4* gene, showing

its resistance overcome. Other cultivars have confirmed their importance as resistance sources and may be used in common bean breeding programs aiming anthracnose.

Obtained results showed resistance/susceptibility patterns discordant of those obtained by Rava et al. (1994); Carneiro (1999); Thomazella et al. (2002); Alzate-Marin and Sartorato (2004); Talamini et al. (2004). Studies revealing cultivars with major susceptibility were Michelite (84.4%), México 222 (81.2%), Cornell 49-242 (46.9%), Perry Marrow (43.7%), Widusa (34.4%), Michigan Dark Red Kidney (31.2%), Kaboon (31.2%), PI 207262 (12.5%) and TO (9.4%). TU, AB 136 and G2333 were resistant to all analyzed isolates in Parana (Sansigolo et al., 2008). Races 3, 10, 11, 15, 27, 31, 63, 75, 79, 81, 82, 83, 90, 91, 93, 95, 259, 283, 287, 339, 346 and 351 showed compatibility reactions with Andean and Mesoamerican cultivars. Similar results were published by Gonçalves-Vidigal et al. (2007).

Comparing obtained data, it is possible to infer that have been occurred changes in the infection patterns observed in the differential cultivars. Damasceno and Silva et al. (2007) characterized isolates of *C. lindemuthianum* in Minas Gerais State and Sansigolo et al. (2008) identified races this fungus in common bean crop in Parana State. It is also important to mention studies conducted by Nunes et al. (2013) and Felipin-Azevedo et al. (2014).

Analysis of reaction pattern in differential cultivars to isolates of *C. lindemuthianum* in this study demonstrates high variability of pathogen races and its expressive spread in producing regions of Parana State. However, the results reveal the importance of constant monitoring of this pathogen occurrence race, by inserting genetic resistance.

CONCLUSIONS

Obtained results enabled the characterization of 25 distinct races of 25 *C. lindemuthianum*, identified in common bean producing Counties in Parana State. This is the first report of races 82, 90, 259, 283, 287, 346 and 351, worldwide, and of races 15, 63 and 339 in Parana State. Race 3 showed the highest occurrence frequency (14.7%).

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