GENETIC VARIABILITY AMONG *Commelina* WEED SPECIES FROM THE STATES OF PARANÁ AND SÃO PAULO, BRAZIL[1]

*Variabilidade Genética entre Espécies de Plantas Daninhas do Gênero **Commelina** Provenientes dos Estados do Paraná e São Paulo, Brasil*

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ABSTRACT - This work aims to carry out a comparative analysis using RAPD molecular markers in four *Commelina* weed species from the state of Paraná and *C. benghalensis* populations from the states of Paraná and São Paulo, Brazil. The genomic plant DNA sample was extracted from the leaves, separated, randomly fragmented and amplified by PCR. Random amplified polymorphic DNA fragments (RAPD markers) were analyzed by using POPGENE statistical program. Eighty-five primer sequences were tested but only three were suitable as molecular markers producing 37 DNA polymorphic fragments for comparisons among four *Commelina* species and 22 polymorphic fragments for comparisons among *C. benghalensis* populations. The results showed that there were inter-specific and intra-specific genetic variabilities among *Commelina* plant genera. Genetic diversity analysis between species indicated four mono-specific clusters and it was suggested to keep *C. villosa* as one species. Regarding the intra-specific genetic variability of *C. benghalensis* alone, three groups were verified, although there were 13 populations from two geographical areas. However, these clusters do not correspond to the distinct characteristics verified.

Keywords: biotype, Commelinaceae, molecular markers, weeds.

*RESUMO - O presente trabalho procurou analisar a variabilidade genética, utilizando-se de marcadores RAPD, comparando quatro espécies de **Commelina** procedentes do Estado do Paraná e entre populações de **C. benghalensis** procedentes dos Estados do Paraná e São Paulo. Foi amostrado o DNA genômico extraído das folhas, o qual foi separado, fragmentado aleatoriamente e amplificado por PCR. Os fragmentos polimórficos de DNA (marcadores RAPD) foram analisados pelo programa estatístico POPGENE. Oitenta e cinco **primers** foram testados, mas somente três sequências foram consideradas marcadores moleculares, produzindo 37 bandas de DNA polimórfico para comparação entre as quatro espécies de **Commelina** e outras 22 para comparação entre as populações de **C. benghalensis**. Os resultados mostraram que há variabilidade genética inter e intraespecífica nas plantas do gênero **Commelina**. A análise da diversidade genética entre as espécies indicou quatro agrupamentos monoespecíficos, e isso sugere manter **C. villosa** como uma espécie. Considerando a variabilidade genética intraespecífica somente de **C. benghalensis**, apesar das 13 populações provenientes de dois Estados geográficos, três grupos foram identificados. Entretanto, esses agrupamentos não correspondem às características morfológicas distintas observadas.*

Palavras-chave: biótipo, Commelinaceae, marcadores moleculares, planta daninha.

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INTRODUCTION

The importance of some members of the Commelinaceae family was emphasized by Holm et al. (1977) since *Commelina* weed plants occur in more than 100 countries in soybean, bean, corn and other cultivated plant crops. Weed species of such family grow in crops and compete for light and nutrients with cultivated plants and might be an alternative host for viruses, fungi and nematodes (Wilson, 1981). The success and spread of *Commelina* weed species in many crops may be attributed to their high environmental adaptation, mainly because their vegetative and seed reproduction systems are highly efficient (Maheshwari & Maheshwari, 1955; Wilson, 1981).

Investigation on the genetic variability of cultivar plants has been conducted for species, variety,cultivar and biotype characterization. Although few studies have been carried out on the genetic diversity of weed plants, some of the more important taxa have been evaluated, such as *Cyperus esculentus* (Horak & Holt, 1986), *Cyperus rotundus* (Silva et al., 2000), and *Commelina benghalensis* (Ahanched & Gasquez, 1992).

In Brazil, four *Commelina* species occur as weed plants, with *C. benghalensis* being considered the most important one because it is the best known, infesting different crops (Silva et al, 2009a, b). However, three other species, *C. diffusa*, *C. erecta* and *C. villosa,* are also important because of their tolerance to glyphosate (Santos, 2002; Rocha et al., 2007).

Some investigations were carried out on the genetic variability of Commelinaceae using enzymatic markers. Ahanched & Gasquez (1992) observed two genetic patterns in *C. benghalensis* populations from Northern to Southern Benin, Africa and Godt & Hamrick (1993) analyzed enzymatic variability among 13 *Tradescantia hirsuticaulis* populations in three US states.

The present work aimed to conduct a comparative analysis using RAPD molecular markers of four *Commelina* weed species naturally occurring from the state of Paraná and matching *C. benghalensis* populations from the states of Paraná and São Paulo, Brazil.

MATERIAL AND METHODS

The genomic DNA from leaves of *C. erecta*, *C. diffusa*, *C. villosa* and *C. benghalensis* populations from Paraná was compared. *C. benghalensis* populations from Paraná and São Paulo, Brazil were also compared (Table 1). The plants were collected from agricultural fields and grown in pots to evaluate their development under greenhouse conditions at the Department of Botany, Biosciences Institute of Botucatu, Universidade Estadual Paulista (UNESP), SP, Brazil.

RAPD analysis protocol was performed as reported by Ferreira & Grattapaglia (1996). Genomic DNA was extracted from matured leaf fragments for maceration in liquid nitrogen. Eighty-five primer DNA sequences (Operon Technologies) were tested, 1 to 20 sequences of primers W, N and P; 2 to 17 sequences of primer J; and 1 to 9 of primer X. Each reaction (final volume 13 μL) had a buffer (10 mM Tris HCl pH 8.0), dNTPs (1.0 mM), primer sequences selected (10 ng μL^{-1}), Taq polimerase (Pharmacia) and around 10 ng of genomic DNA (quantified previously by gel electrophoresis of agarose 0.8%). PCR amplification was conducted in a thermocycler (MJ Research model PTC 100) in 40 cycles. Each cycle was programmed to 1 min at 92 °C, 1 min to 35 °C and 2 min to 72 °C; while the least cycle was increased 5 min to 72 °C.

DNA quantification and DNA amplification were detected after DNA samples were mixed with ethidium bromide and run in agarose gel electrophoresis technique. DNA polymorphism was identified as a band in the autoradiography reported in the photography made with the "Eagle Eyes" program. The band auto radiographs scored as either present (1) or absent (0).

Three sequence primers (W02, W13 and J13) produced 37 DNA polymorphic fragments, for comparisons among four *Commelina* species, and 22 polymorphic fragments, for comparison among *C. benghalensis* populations. Using 1 and 0 as numeric values, two matrices were calculated. Inter-specific and intra-specific clusters were analyzed using the POPGENE program. Two dendrograms were constructed as a result of these analyses.

Table 1 - *Commelina* populations of four weed species from the states of Paraná and São Paulo, Brazil, analysed by RADP molecular markers

Nº	Species	Source	Geographic Coordinates	
01	COMER	Ponta Grossa, PR	S 25 05' 02,3"	W 50 06' 05,7"
02	COMER	Ponta Grossa, PR	S 24 49' 06,5"	W 50 00' 51,7"
03	COMER	Ponta Grossa, PR	S 24 41' 43,1"	W 50 24' 11,0"
04	COMDI	Ponta Grossa, PR	S 25 00' 50,6"	W 50 09' 0,46"
05	COMDI	Ponta Grossa, PR	S 25 05' 49,7"	W 50 06' 28,2"
06	COMDI	Ponta Grossa, PR	S 24 26' 44,0"	W 49 51' 18,5"
07	COMVI	Cascavel, PR	S 25 21' 41,7""	W 53 34' 33,4"
08	COMVI	Ponta Grossa, PR	S 25 01' 51,9"	W 50 09' 42,3"
09	COMVI	Ponta Grossa, PR	S 25 05' 35,0"	W 50 04' 05,3"
10	COMBE	Ponta Grossa, PR	S 25 06' 44,7"	W 50 26' 01,9"
11	COMBE	Ponta Grossa, PR	S 25 08' 51,8"	W 49 58' 35,0"
12	COMBE	Ponta Grossa, PR	S 24 41' 43,1"	W 50 24' 11,0"
13	COMBE	Cascavel, PR	S 24 58' 30,2"	W 53 20' 17,1"
14	COMBE	Cascavel, PR	S 25 13' 44,6"	W 53 35' 22,1"
15	COMBE	Cascavel, PR	S 24 56' 58,4"	W 53 27' 49,3"
16	COMBE	Campo Mourão, PR	S 24 10' 57,5"	W 52 29' 43,9"
17	COMBE	Campo Mourão, PR	S 23 57' 54,3"	W 52 21' 08,9"
18	COMBE	Campo Mourão, PR	S 23 53' 32,0"	W 52 19' 34,5"
19	COMBE	Londrina, PR	S 23 30' 22,1"	W 51 39' 14,5"
20	COMBE	Londrina, PR	S 23 20' 22,9"	W 51 21' 06,9"
21	COMBE	Londrina, PR	S 23 25' 48,0"	W 51 32' 03,9"
22	COMBE	Ourinhos, SP	S 22 55' 14,6"	W 49 54' 13,0"
23	COMBE	Ourinhos, SP	S 22 52' 00,8"	W 49 58' 36,6"
24	COMBE	Ourinhos, SP	S 22 47' 43,7"	W 50 05' 38,4"
25	COMBE	Presidente Prudente, SP	S 22 17' 26,2"	W 51 16' 17,5"
26	COMBE	Presidente Prudente, SP	S 22 11' 56,4"	W 51 21' 15,0"
27	COMBE	Presidente Prudente, SP	S 22 06' 34,0"	W 51 27' 21,4"
28	COMBE	Araçatuba, SP	S 21 09' 34,7"	W 50 13' 42,1"
29	COMBE	Araçatuba, SP	S 21 16' 06,3"	W 50 21' 31,1
30	COMBE	Araçatuba, SP	S 21 21' 12,7"	W 50 18' 35,6"
31	COMBE	São José do Rio Preto,SP	S 20 22' 08,9"	W 49 25' 49,2"
32	COMBE	São José do Rio Preto,SP	S 20 34' 03,8"	W 49 19' 03,8"
33	COMBE	São José do Rio Preto,SP	S 20 25' 40,4"	W 49 25' 29,8"
34	COMBE	Lins, SP	S 21 44' 59,5"	W 49 41' 37,5"
35	COMBE	Lins, SP	S 21 50' 05,6"	W 49 36' 25,0"
36	COMBE	Lins, SP	S 22 03' 49,5"	W 49 24' 09,8"
37	COMBE	Ribeirão Preto, SP	S 21 19' 10,9"	W 47 47' 44,7"
38	COMBE	Ribeirão Preto, SP	S 21 22' 03,1"	W 47 42' 52,9"
39	COMBE	Ribeirão Preto, SP	S 21 22' 05,8"	W 47 50' 56,4"
40	COMBE	Mogi Mirim, SP	S 22 21' 18,2"	W 47 11' 00,2"
41	COMBE	Mogi Mirim, SP	S 22 23' 55,3"	W 47 17' 42,5"
42	COMBE	Mogi Mirim, SP	S 22 24' 32,2"	W 47 04' 53,2"
43	COMBE	Botucatu, SP	S 22 53' 00,0"	W 48 26' 22,6"
44	COMBE	Botucatu, SP	S 22 53' 11,4"	W 48 29' 50,7"
45	COMBE	Botucatu, SP	S 22 47' 50,1"	W 48 32' 38,7"
46	COMBE	Araraquara, SP	S 21 39' 28,5"	W 48 22' 21,6"
47	COMBE	Araraquara, SP	S 21 42' 51,2"	W 48 18' 30,8"
48	COMBE	Araraquara, SP	S 21 44' 44,6	W 48 16' 12,8

COMER: *C. erecta*. COMDI: *C. diffusa*. COMVI: *C. villosa*. COMBE: *C. benghalensis*.

RESULTS AND DISCUSSION

Only three (2.55%) of the 85 sequence primers used to test amplified *Commelina* genomic DNA indicated low polymorphism rate. However, this is a preliminary DNA analysis in *Commelina* weeds, indicating genetic diversity between species, as four mono-specific clusters were constructed using statistical analysis. This may be an important contribution to taxonomic evaluations, corroborating with morphological studies on *Commelina* species identification (Rocha et al., 2007).

Amplified DNA fragments after PCR reaction using sequence primer J13 are shown in Figure 1. There are different band patterns among four *Commelina* species (1 to 12) from Paraná. However, there are similarities among *C. benghalensis* species, although there are 13 populations from the states of Paraná and São Paulo (10 to 48).

The first dendrogram shown in Figure 2A is the result of the statistical analysis using 37 molecular markers produced by PCR with three sequences primers to four *Commelina* species from Paraná. There has been a controversy about the taxonomic position of *C. villosa* as to whether this taxon is another species or a variety of *C. erecta* (Barreto, 1997).The statistical analysis of the present RAPD markers shows that the genetic standard of *C. villosa* is more similar to that of *C. diffusa* and *C. benghalensis* than to that of *C. erecta* (Figure 2A). These results will help further the taxonomic studies of *Commelina* and reinforce the concept that *C. villosa* is a species.

The second dendrogram (Figure 2B) was constructed to compare the intra-specific genetic variability of *C. benghalensis* alone, using the same statistical analysis of the other 22 molecular markers of the 13 populations from the states of Paraná and São Paulo, with the three main groups (GI, GII, GIII) being observed and within each group, with other similarity groups being detected. However, morphological characteristics that justified these three genetic clusters were not detected.

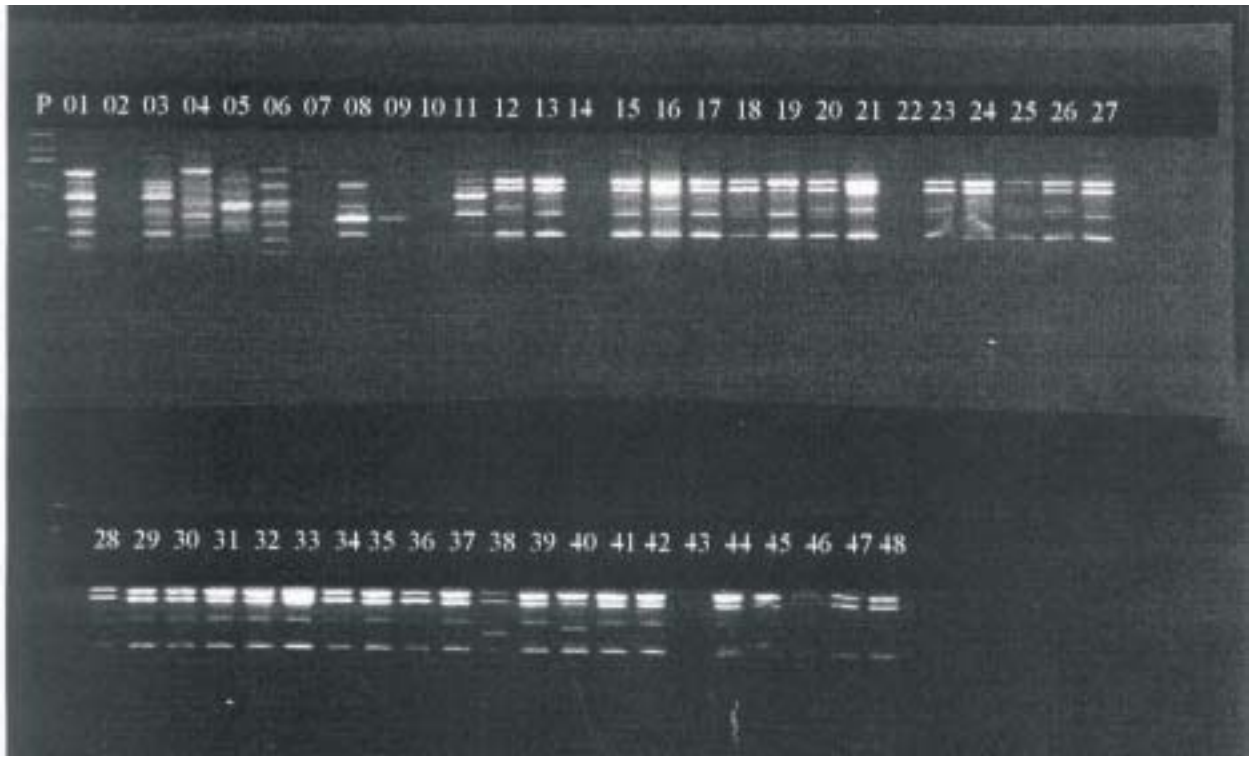

Figure 1 - RAPD markers obtained by fragmented genomic DNA from *Commelina* populations, after reaction with J13 sequence primer. Pattern (P). *C. erecta* (01-03), *C. diffusa* (04-06), *C. villosa* (07-09), *C. benghalensis* populations from Paraná, Brazil: Ponta Grossa (10-12), Cascavel (13-15), Campo Mourão (16-18), Londrina (19-21); and *C. benghalensis* populations from São Paulo, Brazil: Ourinhos (22-24), Presidente Prudente (25-27), Araçatuba (28-30), São José do Rio Preto (31-33), Lins (34-36), Ribeirão Preto (37-39), Mogi Mirim (40-42), Botucatu (43-45) and Araraquara (46-48).

Considering only the morphology of *C. benghalensis*, three samples from Paraná (Cascavel, Ponta Grossa and Campo Mourão) and two samples from São Paulo (Presidente Prudente and Araçatuba) were collected displaying leaves without pilosity. Leaf pilosity is cited as an important morphological feature of *C. benghalensis* species (Rocha et al., 2007). Other morphological characteristics were also observed between these plants indicating two phenotypical standards, although a corresponding genomic variability was not observed. Morton (1967) verified these two morphological leaf structures of *C. benghalensis* in the wet African mountains and classified them as two subspecies: *C. benghalensis* subsp. *benghalensis,* without leaf pilosity and *C. benghalensis* subsp. *hirsute*, with intense leaf pilosity. Lorenzi (2000) presents illustrations, though not citing the two subspecies classification, noting that leaf structure is the only difference between them.

The geographic distribution only of the *C. benghalensis* populations studied is shown in Figure 3. While low genetic similarity seems to exist among the four Paraná populations, all the São Paulo populations were congregated in one group. There were no samples of *C. benghalensis* from southern Parana (latitude below 25°) as this species was not found there. Some genotypic standards were observed among the four Paraná populations. Group I was formed with Ponta Grossa and Campo Mourão of *C. benghalensis* populations which are more similar. Londrina (Paraná) populations presented more similarities with other São Paulo populations, constituting Group II. *C. benghalensis* populations from Cascavel were isolated to form Group III as their genetic patterns are more different than that of other populations

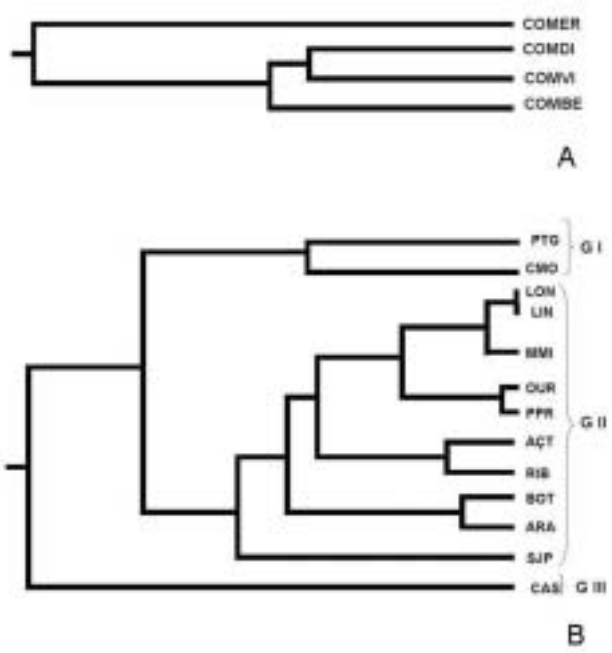

Figure 2 - Dendrograms produced by Cluster Analysis using RAPD molecular markers. **A**. Four *Commelina* weed plant from Paraná, Brazil. COMBE: *C. benghalensis*. COMDI: *C. diffusa*. COMER: *C. erecta*. COMVI: *C. villosa*. **B**. *C. benghalensis* populations from Paraná and São Paulo, Brazil. AÇT: Araçatuba. ARA: Araraquara. BOT: Botucatu. CAS: Cascavel. CMO: Campo Mourão. LIN: Lins. LON: Londrina. MMI: Mogi Mirim. OUR: Ourinhos. PPR: Presidente Prudente. PTG: Ponta Grossa. RIB: Ribeirão Preto. SJP: São José do Rio Preto.

from Paraná. Again, this segregation does not match the morphological characteristics. *C. benghalensis* has autogamic reproduction (Maheshwari & Maheshwari, 1955; Wilson, 1981) and this could be the cause of the low gene flow among the populations from Paraná.

However, climatic conditions also might explain this group arrangement, despite the distance between local populations and geographical boundaries. There is more similarity between the climatic conditions of northern Paraná and those of São Paulo than with other Paraná regions (IBGE, 1977). Northern Paraná's climate, relief and natural vegetation are more similar to those in the state of São Paulo and *C. benghalensis* populations from Londrina probably present more similarity with *C. benghalensis* populations from São Paulo because they are adapted to these environmental conditions.

There is also a difference in agricultural methods applied by farmers in the Paraná regions. No-tillage cropping system has been used in central and southern Paraná regions since the 1970s, aiming to decrease weed infestation and reduce the need of herbicide control (Pauletti & Seganfredo, 1999; Gomes & Christoffoleti, 2008). However, this technique is not commonly applied in the northern Paraná regions and in the state of São Paulo. Ahanched & Gasquez (1992) verified similar results, citing a significant genetic variability among *C. benghalensis* populations from Southern and Northern Benin-Western Africa. These authors conducted enzymatic polymorphism analyses and concluded that the genetic variability observed was due to the agricultural techniques applied. In another study using enzymatic polymorphism analysis with Commelinaceae species, Godt & Hamrick (1993) verified genetic similarities among *Tradescantia hirsuticaulis* populations from Alabama, Georgia and South Carolina. These results are similar to the data presented in this work, as cluster formation surpassed geographic boundaries and a low gene flow occurred in great distances. Considering that the reproduction system of Commelinaceae species presents asexual strategies (Maheshwari & Maheshwari, 1955; Wilson, 1981; Gamerro, 1986), their genomics is likely being preserved.

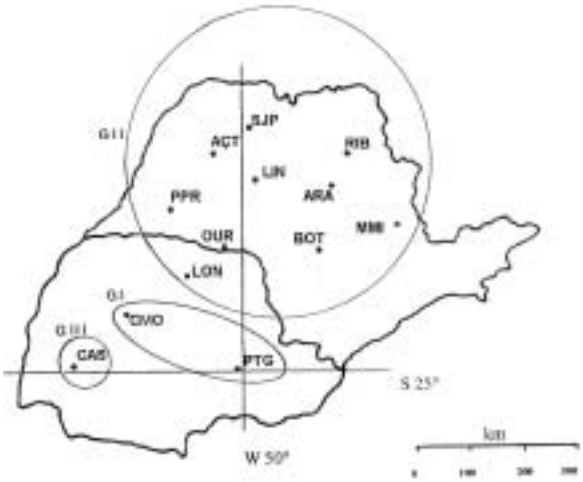

Figure 3 - *Commelina benghalensis* geographic population from Paraná and São Paulo, Brazil, clustered using 22 RAPD molecular markers. AÇT: Araçatuba. ARA: Araraquara. BOT: Botucatu. CAS: Cascavel. CMO: Campo Mourão. LIN: Lins. LON: Londrina. MMI: Mogi Mirim. OUR: Ourinhos. PPR: Presidente Prudente. PTG: Ponta Grossa. RIB: Ribeirão Preto. SJP: São José do Rio Preto.

On the other hand, genetic similarity among accesses of *Avena sterilis* were studied by Heun et al. (1994) who conducted a comparative analysis using RAPD molecular and enzymatic markers, and concluded that RAPD markers were highly efficient in separating the 24 biotypes tested. McCloskey & Holt (1990) also reported genetic differences between two biotypes of *Senecio vulgaris* (one resistant and the other susceptible to the herbicide triazine) using molecular analysis with RAPD technique. Preliminary genetic variability analysis among species and clones of *Populus* plants were observed by Sanchez et al. (1998), using only one sequence primer. The present study is similar, although using a few primer sequences.

RAPD molecular markers did not reveal specific gene information in this work, but further research and more complex studies must be conducted to identify more efficient molecular markers that can be used to separate different genetic clusters within weed species as it has been already done with cultivated plants by Chen et al. (1998) and Pejic et al. (1998).

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