CYTOGENETIC DATA FOR *Paspalum notatum* FLÜGGE ACCESSIONS

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ABSTRACT: Several species of the genus *Paspalum* L. are important forages, due to quality, productivity and tolerance to environmental stresses. Chromosome numbers, meiotic configurations and pollen fertility were evaluated in a collection of 85 accessions of *Paspalum notatum* Flügge and in seven accessions of Pensacola (*P. notatum* var *saurae*). All *P. notatum* accessions were tetraploid, with 2n = 4x = 40, except one diploid accession, considered as an escape of Pensacola. All Pensacola plants had 2n = 2x = 20. Meiotic configurations at diakinesis and metaphase I varied among tetraploid accessions, from plants with only bivalents to plants with high frequency of quadrivalents. Pollen fertility varied from 82.5 to 95.9% among diploid accessions and from 72.4 to 97.9% among the tetraploid. Due to the apomictic mode of reproduction of tetraploid *P. notatum*, meiotic irregularities can be maintained by the plants without harming their propagation. At the same time, pollen fertility should be high enough to assure endosperm development, since the species is pseudogamous. Wild diploid *P. notatum* populations, apart from the endemic *P. notatum* var *saurae* are very rare. From a plant breeding point of view, all the examined tetraploid accessions are potentially male-fertile and could be used as males in crosses.
Key words: apomixis, chromosome numbers, meiotic configurations, plant breeding, pollen fertility

DADOS CITOGENÉTICOS PARA ACESSOS DE *Paspalum notatum* FLÜGGE

RESUMO: Muitas espécies do genus *Paspalum* L. são importantes forragens, devido à qualidade, produtividade e tolerância para o stress ambiental. Números cromossômicos, configurações meióticas e fertilidade do pólen foram analisados em uma coleção de 85 acessos de *Paspalum notatum* Flügge e sete acessos de Pensacola (*P. notatum* var *saurae*). Todos os acessos de *P. notatum* eram tetraplóides, com 2n = 4x = 40, com exceção de um diplóide, considerado como escape de Pensacola. Todas as plantas de Pensacola examinadas tinham 2n = 2x = 20. Foi verificada variação entre os acessos tetraplóides quanto às configurações cromossômicas em diacinese e metáfase I, desde plantas com apenas bivalentes até aquelas com alta freqüência de quadrivalentes. A fertilidade do pólen variou de 82,5 a 95,9% entre os acessos diplóides e de 72,4 a 97,9% entre os tetraplóides. Devido ao modo de reprodução apomítico de *P. notatum* tetraplóide, irregularidades meióticas podem ser mantidas sem prejuízo da propagação. Ao mesmo tempo, a fertilidade do pólen deve ser suficientemente alta para assegurar a formação do endosperma, já que a espécie é pseudogâmica. Diplóides silvestres de *P. notatum*, além do endêmico *P. notatum* var *saurae* são muito raros. Do ponto de vista do melhoramento, todos os tetraplóides são potencialmente macho-férteis e poderiam ser utilizados como genitores masculinos em cruzamentos.
Palavras-chave: apomixia, números cromossômicos, configurações meióticas, melhoramento genético, fertilidade do pólen

INTRODUCTION

The genus *Paspalum* L. comprises around 400 species, most of them native to tropical and sub-tropical Americas (Moraes-Fernandes et al., 1968). Many of these species are important forages, due to quality, productivity and tolerance to environmental stresses (Valls, 2000). In Brazil, where 75% of *Paspalum* species occur, they are part of several plant communities, in a broad range of ecological conditions (Barreto, 1974). Sexual reproduction and apomixis are common in the genus, generally related to the diploid and polyploid levels (Quarín & Norrmann, 1990).

P. notatum Flügge is an apomictic tetraploid (2n = 4x = 40), common to native pastures along warm and temperate American regions, from Central

Mexico to Uruguay (Burton, 1948; Burton & Forbes, 1960; Quarín et al., 1984) and has been studied by botanists, geneticists, agronomists and plant breeders (Dahmer-Balbinot, 2007). *P. notatum* var. *saurae* is a sexual diploid (2n = 2x = 20), native to northeastern and central-eastern Argentina, introduced around 1926 to the Pensacola Bay region of Florida and later released as the Pensacola cultivar (Burton, 1967; 1974). It is widely cultivated in North and South America. Since the apomictic nature of *P. notatum* precludes its use as female in crosses (but not as male given there is fertile pollen), the introduction of variability by sexual methods was possible by crossing the apomictic with artificially polyploidized *P. notatum* var. *saurae* (Burton & Forbes, 1960; Burton et al., 1970). If other wild diploids were identified, they could have been used to introduce novel variation in *P. notatum*, but, until now, no other diploids have been located outside the Argentinian region of *P. notatum* var. *saurae* (Pozzobon & Valls, 1997; Daurelio et al., 2004). The objective of the present work was to determine chromosome numbers, chromosome associations at diakinesis and metaphase I, and pollen viability in accessions of *P. notatum* which are part of a breeding project.

MATERIAL AND METHODS

The original plants (accessions) were collected as tillers (propagated later vegetatively in pots) from populations in the Rio Grande do Sul State, Brazil. A few accessions came from the Santa Catarina and Paraná States (Brazil), Uruguay and Argentina (Table 1). Plants are kept in pots as part of a germplasm collection. The accessions are identified in the collection by species name and a number, for working purposes. In the collection list all accessions are registered by this number plus the collector´s number and place of collection.

Table 1 - List of *P. notatum* accessions, chromosome number, chromosome associations and pollen viability.

Accession number		Collector's number[a]	Collection site[b]	2n[c]	Chromosome associations[d]	Pollen viability %
P. notatum	01	Valls 14244A	Uruguaiana, RS	40	-	
P. notatum	02	Valls 14244E	Uruguaiana, RS	40	-	86.53
P. notatum	03	Valls 14244H	Uruguaiana, RS	40	-	82.80
P. notatum	04	Valls 14310	Barra do Quaraí, RS	40	20II (20) 18II + 1IV (7)	96.00
P. notatum	05	Valls 14326	Capivari do Sul, RS	40	20II (28) 18II + 1IV (5) 14II + 3IV (2)	74.33
P. notatum	06	Valls 14329	Capivari do Sul , RS	40	20II (9)	83.87
P. notatum	07	Valls 14327	Capivari do Sul , RS	40	20II (19)	72.40
P. notatum	08	Valls 14314	Itaquiraí , MS	40	20II (13) 18II + 1IV (3) 14II + 3IV (2)	88.40
P. notatum	10	Valls 14827	Candói , PR	40	-	97.93
P. notatum	11	Valls 14828	Candói , PR	40	18II + 1IV (11) 16II + 2IV (8) 2II + 6IV (2)	85.80
P. notatum	12	Valls 14829	Candói , PR	20	10II (26) 8II + 1 IV (2)	91.20
P. notatum	13	Valls 14865	Capivari do Sul, RS	40	20II (23)	82.53
P. notatum	14	Valls 14866	Capivari do Sul , RS	40	20II (6) 18II + 1IV (10) 16II + 2IV (5) 8II + 6IV (3)	84.47
P. notatum	16	Valls 14869	Capivari do Sul , RS	40	20II (35) 18II + 1IV (2)	97.40
P. notatum	17	Valls 14870	Capivari do Sul , RS	40	20II (45)	91.07
P. notatum	18	Valls 14871	Capivari do Sul , RS	40	20II (65) 18II + 1IV (2)	90.07
P. notatum	23	MD s/n	Eldorado do Sul , RS	40	-	
P. notatum	24	MD s/n	Eldorado do Sul , RS	40	-	

Continue...

Table 1 - Continuation.

P. notatum	25	MD s/n	Lavras do Sul, RS	40	20II (15) 18II + 1IV (4) 16II + 2IV (2)	86.53
P. notatum	26	MD s/n	Lavras do Sul,RS	40	20II (5) 18II + 1IV (9) 16II + 2IV (3)	82.80
P. notatum	27	MD s/n	Lavras do Sul, RS	40	20II (7) 18II + 1IV (3) 16II + 2IV (4) 14II + 3IV (2)	96.00
P. notatum	28	MD s/n	Caçapava do Sul , RS	40	20II (17) 18II + 1IV (3)	74.33
P. notatum	29	MD s/n	Caçapava do Sul , RS	40	20II (18) 18II + 1IV (8) 16II + 2IV (7) 14II + 3IV (5)	83.87
P. notatum	30	MD s/n	Bagé, RS	40	20II (17) 18II + 1IV (2) 16II + 2IV (5)	72.40
P. notatum	31	MD s/n	André da Rocha, RS	40		88.40
P. notatum	32	MD s/n	Barretos, SP	40	20II (21) 18II + 1IV (3)	82.20
P. notatum	33	MD s/n	Vacaria , RS	40	20II (2) 18II + 1IV (5) 14II + 3IV (3) 16II + 2IV (19)	97.93
P. notatum	34	MD s/n	Vacaria , RS	40	-	85.80
P. notatum	35	MD s/n	São Borja, RS	40	20II (19) 16II + 2IV (2)	91.20
P. notatum	36	CN s/n	Arapeí 01, San Tomé, Argentina	40	-	82.53
P. notatum	37	CN s/n	Arapeí 02, San Tomé, Argentina	40	20II (33) 18II + 1IV (3) 17II + 3I + 1III (7) 16II + 2IV (5) 14II + 3IV (4) 10II + 5IV (2)	84.47
P. notatum	38	CN s/n	Arapeí 03, San Tomé, Argentina	40	-	97.40
P. notatum	39	CN s/n	Arapeí 04, San Tomé, Argentina	40	-	91.07
P. notatum	41	CN s/n	Arapeí 06, San Tomé, Argentina	40	20II (7) 18II + 1IV (10) 16II + 2IV (2)	-
P. notatum	42	CN s/n	Arapeí 07, San Tomé, Argentina	40	20II (19) 18II + 2IV (3)	-
P. notatum	43	CN s/n	Arapeí 08, San Tomé, Argentina	40	-	-
P. notatum	44	CN s/n	Arapeí 09, San Tomé, Argentina	40	20II (11) 18II + 1IV(3) 17II + 3I + 1III (2) 16II + 2IV (2) 10II + 5IV (2)	-
P. notatum	46	CN s/n	Arapeí 11, San Tomé, Argentina	40	20II (17) 18II + 1IV (3) 16II + 2IV (5)	83.27
P. notatum	47	CN s/n	Arapeí 12, San Tomé, Argentina	40	-	89.20

Continue...

Table 1 - Continuation.

P. notatum	49	MD s/n	Mostardas, RS	40	20II (42)	91.87
P. notatum	50	CN s/n	Piracicaba , SP	40	20II (16) 18II + 1IV (6) 12II + 4IV (3)	90.00
P. notatum	51	MD s/n	Possadas , Argentina	40	-	73.00
P. notatum	52	Steiner s/n	Santiago, RS	40	-	89.53
P. notatum	53	MD s/n	Água Doce, SC	40	-	-
P. notatum	54	MD s/n	Eldorado do Sul, RS	40	20II (32) 18II + 1IV (2) 16II + 2IV (10)	84.93
P. notatum	55	Valls 14921	Quarai, RS	40	20II (4) 18II + 1IV (2) 16II + 2IV (4) 14II + 3IV (10) 12II + 4IV (4)	81.8
P. notatum	56	Valls w14931	Alegrete, RS	40	20II (12) 18II + 1IV (2) 16II + 2IV (2) 14II + 3IV (2) 10II + 5IV (4)	-
P. notatum	66	MD s/n	Uruguai	40	20II (15) 16II + 2IV (9)	88.93
P. notatum	67	MD s/n	Uruguai	40	20II (12) 18II + IV (22) 16II + 2IV (7)	89.73
P. notatum	69	MD s/n	São José do Hortêncio, RS	40	20II (12) 18II + 1IV (4) 16II + 2IV (13)	76.93
P. notatum	70	ND s/n	Porto Lucena, RS	40	-	91.00
P. notatum	71	ND s/n	Três de Maio, RS	40	-	-
P. notatum	72	ND s/n	Três de Maio, RS	40	-	-
P. notatum	73	ND s/n	Cruz Alta, RS	40	-	-
P. notatum	74	MD & CN s/n	Tapes, RS	40	-	-
P. notatum	75	MD & CN s/n	Cristal , RS	40	-	-
P. notatum	76	MD & CN s/n	São Lourenço do Sul,RS	40	-	-
P. notatum	77	MD & CN s/n	Capão do Leão, RS	40	-	-
P. notatum	78	MD & CN s/n	Capão do Leão, RS	40	-	-
P. notatum	79	MD & CN s/n	Pinheiro Machado, RS	40	-	-
P. notatum	80	MD & CN s/n	Pinheiro Machado, RS	40	-	-
P. notatum	81	MD & CN s/n	Pinheiro Machado, RS	40	-	-
P. notatum	82	MD & CN s/n	Candiota, RS	40	-	-
P. notatum	83	MD & CN s/n	Hulha Negra, RS	40	-	-
P. notatum	84	MD & CN s/n	Bexigoso, RS	40	-	-
P. notatum	85	MD & CN s/n	Dom Pedrito, RS	40	-	-
P. notatum	86	MD & CN s/n	Dom Pedrito, RS	40	-	-
P. notatum	87	MD & CN s/n	Dom Pedrito, RS	40	-	-
P. notatum	88	MD & CN s/n	Livramento, RS	40	-	-
P. notatum	89	MD & CN s/n	Livramento, RS	40	-	-
P. notatum	90	MD & CN s/n	Livramento, RS	40	-	-
P. notatum	92	MD & CN s/n	Areial, RS	40	-	-
P. notatum	93	MD & CN s/n	Areial, RS	40	-	-

Continue...

Table 1 - Continuation.

P. notatum	95	MD & CN s/n	Alegrete, RS	40	-	-
P. notatum	96	MD & CN s/n	Alegrete, RS	40	-	-
P. notatum	97	MD & CN s/n	Alegrete, RS	40	-	-
P. notatum	98	MD & CN s/n	Alegrete, RS	40	-	-
P. notatum	100	MD & CN s/n	Rosário do Sul, RS	40	-	-
P. notatum	101	MD & CN s/n	São Gabriel, RS	40	-	-
P. notatum	102	MD & CN s/n	São Gabriel, RS	40	-	-
P. notatum	105	MD & CN s/n	Vila Nova do Sul, RS	40	-	-
P. notatum	106	MD & CN s/n	Vila Nova do Sul, RS	40	-	-
P. notatum	673	MD & CN s/n	Eldorado do Sul, RS	40	-	-
P. notatum Bagual[e]	01	CN s/n	Região das Missões, RS	40	20II (11) 18II + 1IV (6) 12II + 4IV (2) 10II + 5IV (12) 8II + 6IV (10) 6II + 7IV (5)	86.33
P. notatum André da Rocha[e]	01	CN s/n	André da Rocha, RS	40	20II (26) 18II + 1IV(12) 16II + 2IV (6) 140II + 3IV (12) 12II + 4IV (3) 10II + 5IV (6)	91.42
Pensacola	02	MD s/n	Viamão, RS	20	10II (21)	94.00
Pensacola	03	MD s/n	Viamão, RS	20	10II (33)	88.60
Pensacola	04	MD s/n	Viamão, RS	20	-	87.27
Pensacola	05	MD s/n	Viamão, RS	20	10II (23)	89.73
Pensacola	06	MD s/n	Viamão, RS	20	10II (40)	92.53
Pensacola	09	MD s/n	Viamão, RS	20	10II (35)	82.47
Pensacola	10	MD s/n	Viamão, RS	20	10II (35)	95.93

[a]Valls: José Francisco M. Valls, EMBRAPA-CENARGEN, Brasília, DF; MD:- Miguel Dall'Agnol, UFRGS, Porto Alegre, RS; CN: Carlos Nabinger, UFRGS, Porto Alegre, RS; Steiner: Marcelo Steiner, UFRGS, Porto Alegre, RS; ND: Nair Dahmer, UFRGS, Porto Alegre, RS; [b]RS: Rio Grande do Sul; PR: Paraná; SC: Santa Catarina; SP: São Paulo; MS: Mato Grosso do Sul; [c]To standardize the results, chromosome numbers are all represented as 2n; [d]Between brackets is the number of analyzed cells; [e]Bagual and André da Rocha are informal denominations for two *P. notatum* populations with particular characteristics

Somatic chromosome numbers (2n) were determined in root-tip cells pre-treated with a saturated paradichlorobenzene solution for 24 h at 4°C, fixed in Carnoy 3:1 (ethanol:acetic acid) for 24 h at 4°C, hydrolyzed with 1 mol L^{-1} HCl for 10 min at 60ºC, stained with Feulgen and squashed in propionic carmine. At least ten cells with good spreading and no chromosome overlapping were counted per plant. Gametic chromosome numbers (n) were determined in pollen-mother-cells undergoing meiosis, from young inflorescences fixed in Carnoy 3:1 at room temperature and squashed in propionic carmine. Most of the gametic chromosome counts were performed in cells at diakinesis and metaphase I stages, since at these stages also chromosome pairing configurations may clearly be observed.

Pollen fertility was estimated by stainability (in 2% propionic carmine, following Simioni et al., 2006) of 1500 mature pollen grains per plant. Full, well stained grains were classified as potentially viable and unstained or poorly stained as sterile.

RESULTS AND DISCUSSION

Chromosome numbers were determined in a total of 92 accessions (Table 1), and, 83 of them were tetraploid, with 2n = 4x = 40 (or n = 20) (Figures 1A, B, C). One accession, originally collected as *P. notatum* had 2n = 2x = 20 (*P. notatum* 12) (Table 1, Figures 1G, H). The seven Pensacola accessions were diploid, with 2n = 2x = 20 (or n = 10), as expected (Table 1).

The *P. notatum* 12 accession with 2n = 20 is most probably an escape of cultivated Pensacola. Despite having been collected among plants of *P. notatum*, Pensacola is cultivated in the region and most probably some plants of the cultivated variety have invaded the natural fields.

Pozzobon & Valls (1997) reported 11 accessions with 2n = 20 among the 127 *P. notatum* accessions and considered these plants to be escapes from Pensacola cultivation. Moraes-Fernandes et al. (1973) found only the tetraploid level among the 16 Brazilian (Rio Grande do Sul) and one introduced accession from *P. notatum*. Daurelio et al. (2004) reported that despite a search in other regions of Argentina, the diploids are all botanically *P. notatum* var. *saurae* and restricted to Northeastern and Central-Eastern Argentina. In this restricted region, but not further, these authors detected some triploid hybrids between *P. notatum* and *P. notatum* var. *saurae*.

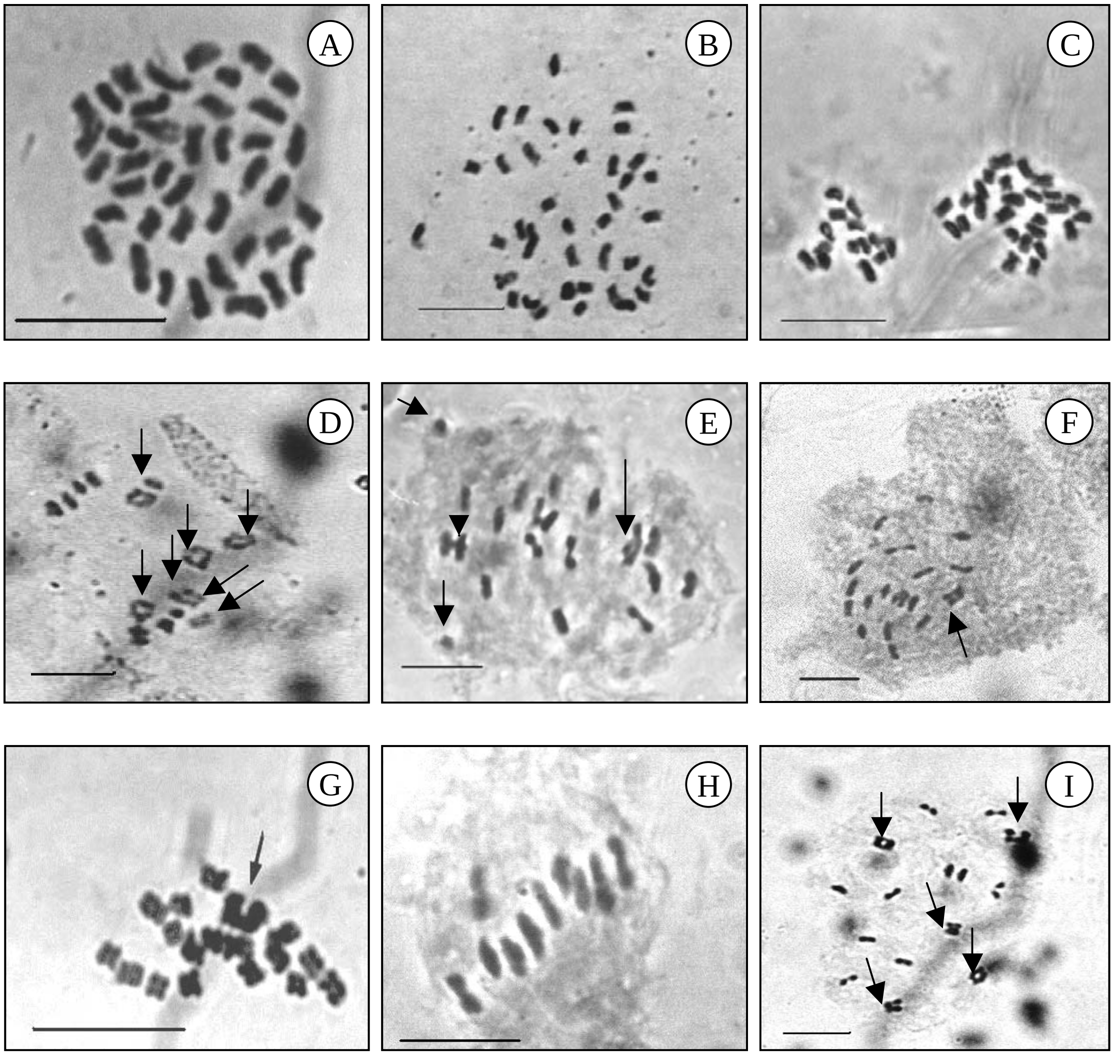
Wild diploid cytotypes are most probably restricted to this specific Argentinian region. If "true" diploid *P. notatum* plants, apart from Pensacola and its escapes exist in other geographical areas they are certainly extremely rare. To find other possible wild diploids in other regions would imply in collecting a high number of accessions along *P. notatum* distribution and would require other methods than traditional chromosome countings (that are laborious and time consuming) to estimate ploidy levels, such as flow cytometry that allows examination of a large number of plants in less time. However, once Pensacola is also widely cultivated, these studies should be coupled to a sound taxonomic determination, detailed morphological analysis and, if possible other approaches as molecular markers profile, in other to avoid misidentifications and wrong assignments of Pensacola escapes as wild *P. notatum*.

Despite the absence of chromosome number variability among the *P.notatum* accessions, when examining meiotic configurations (Table 1), the 36 tetraploid accessions presented several chromosome configurations, with the occurrence of univalents (I), bivalents (II), trivalents (III), and quadrivalents (IV) (Table 1, Figures 1E, F). The frequencies of different chromosome configurations varied widely among different accessions. In *P. notatum* 06, *P. notatum* 07, *P. notatum* 13, *P. notatum* 17 and *P. notatum* 49 all the cells presented only bivalents. On the other hand, in *P. notatum* 14, 75.00% of the cells analyzed presented other configurations than bivalents, and these percentages were 93.10% for *P. notatum* 33, 80.95% for *P. notatum* Bagual and 85.00% for *P. notatum* André da Rocha. In general, the most common configurations among cells were 20 II or 18 II and 1 IV (Table 1). Therefore, the absence of chromosome number variability is accompanied by the variability in meiotic configurations among the tetraploid accessions. Conversely, the diploid accessions presented always ten bivalents at diakinesis and metaphase I (7% of the cells of accession 12) (Table 1, Figures 1H, I). Moraes-Fernandes et al. (1973) also found varying chromosome configurations among the examined accessions of *P. notatum*, with predominance of quadrivalents and bivalents, while trivalents and univalents were less frequent. One accession presented a predominance of cells with just bivalents (Moraes-Fernandes et al., 1973). Variability in chromosome configurations in other *Paspalum* species have been reported by other authors such as Moraes-Fernandes et al. (1968), Pagliarini et al. (2001) and Adamowski et al. (2005).

Pollen viability was mostly over 80% in 48 accessions and ranged from 82.47 to 95.93% among diploid accessions and from 72.40 to 97.93% among tetraploids (Table 1). Moraes-Fernandes et al. (1973) reported pollen fertility (ascertained with acetic carmine) ranging from 0 to 84.3% in tetraploid *P. notatum*.

The variability observed among the *P. notatum* accessions may be explained by the polyploid nature of the species and by its mode of reproduction. Tetraploid *P. notatum* is apomictic and also propagates vegetatively, therefore the quadrivalents remaining from the polyploidization evolutionary process could be maintained (without need for chromosome "diploidization", a process known to occur widely among sexual tetraploids). Moreover, the eventual chromosomal rearrangements that lead to quadrivalents, such as translocations, or other configurations like trivalents and unpaired chromosomes (univalents) could be maintained by the accessions without harming their assexual reproduction. At the same time, since *P. notatum* is a pseudogamous apomictic, that is, needs pollination to form the endosperm, a certain degree of meiotic regularity leading to at least some pollen fertility is necessary and that would explain the relatively high pollen fertility that was observed. It could be speculated that this kind of situation could represent a genetic and evolutionary conflict: apomictic reproduction with the possibility of accumulating meiotic irregularities versus the need of fertile pollen.

From a plant breeding point of view, all the examined accessions are potentially male-fertile and could be used as male genitors in crosses. However, as no other diploids apart from the endemic *P. notatum* var. *saurae* have been identified, improvement of tetraploid *P. notatum* should rely on the selection of well adapted apomictic populations. To introduce variation through sexual crosses would imply in looking for wild *P. notatum* diploid cytotypes, to use other plants of *P. notatum* var. *saurae* from Argentina or even to employ



Figures 1 - (A) *P. notatum* André da Rocha, somatic metaphase (2n = 40); (B) *P. notatum* 87, somatic metaphase (2n = 40); (C) *P. notatum* 13, somatic metaphase (2n = 40); (D) *P. notatum* Bagual, metaphase I (6II + 7IV), arrows point to the quadrivalents; (E) *P. notatum* 05, metaphase I (2I + 17II + 1IV), small arrows point to the univalent, arrow head to secondary associations between two bivalents and the big arrow to the quadrivalent; (F) *P. notatum* 66, metaphase I (18II + 1IV), arrow point to the quadrivalent; (G) *P. notatum* 12, somatic metaphase (2n = 20), arrow points to overlapping of chromosomes; (H) *P. notatum* 12, metaphase I (10 II); (I) *P. notatum* 56, metaphase I (10II + 5IV), arrows point to the quadrivalents. Scale bar = 10 µm.

Pensacola plants. For this the plant breeder would however need to evaluate which new characteristics to be looking for and if these characteristics are found among the known diploid material.

CONCLUSIONS

Wild diploid cytotypes, apart from *P. notatum* var. *saurae* are extremely rare. Despite no variability was found in chromosome numbers among tetraploid *P. notatum*, there is a variability in meiotic chromosome associations among accessions, explained by the apomictic kind of reproduction. Pollen fertility among apomicts is relatively high, assuring endosperm formation and allowing the potential use of these plants as males in crosses**.**

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