

Meiotic studies of some South African cultivars of *Lantana camara* (Verbenaceae)

J. J. SPIES* and C. H. STIRTON**

ABSTRACT

Lantana camara L. is a polyploid species with a basic chromosome number of 11 ($x=11$). Chromosome association in 39 cultivars indicated the occurrence of univalents to heptavalents with bivalents predominating. Multivalent association analysis revealed the presence in South Africa of at least four different groups of *L. camara* at the diploid level.

The potential for sexual reproduction must exist, at least at the diploid level, to account for differences in chromosomal behaviour that can only be attributed to hybridization. The possibility exists that the basic chromosome number may be lower than 11, or else postspeciation genomic evolution must have occurred. No cytogenetical correlation exists between the South African and Indian cultivars.

The number of chiasmata per genome increases with an increase in the polyploid level. Most multivalents are of the chain type. Univalents during diakinesis are the result of asynapsis. Triploid and pentaploid plants display a markedly abnormal meiosis. *L. camara* is a segmental allopolyploid species.

INTRODUCTION

Lantana camara L. is a widespread tropical weed noted for its toxicity to livestock and for its rapid invasion of natural pasturage, waste ground, forest margins and derelict or cultivated lands.

Experience gained from a number of areas of the tropics over many decades has shown that *L. camara* is not a homogeneous species, but actually consists of a number of forms. The origin, nature and spread of these forms have been discussed in the first paper of this series (Stirton, 1977).

L. camara is today recognized as a polyploid aggregate species with a basic chromosome number of $x=11$ and having diploid, triploid, tetraploid, pentaploid and hexaploid representatives (Schnack & Covas, 1947; Tjio, 1948; Singh, 1951; Sen & Sahni, 1955; Tandon & Bali, 1955; Tandon & Chandi, 1955; Natarajan & Ahuja, 1957; Henderson, 1969; Spies & Stirton, 1982). The need for an understanding of these different forms and their taxonomic relationships in South Africa prompted the biological control section of the Department of Agriculture and Fisheries to approach the Botanical Research Institute, of the same Department, to initiate a taxonomic study of *L. camara* in South Africa. This paper reports in depth on the value of meiotic analysis in circumscribing the more than 50 naturalized cultivars of the weed that are presently recognized in South Africa (Stirton & Spies, 1982).

MATERIALS AND METHODS

The plants used in this study are naturalized cultivars collected throughout South Africa and transplanted under quarantine in the Pretoria National Botanical Garden. Thirty-nine different cultivars, representative of all polyploid levels, were used in this study, 37 specimens being sampled from a hybrid swarm from the Transvaal lowveld.

Young inflorescences were collected between 09h00 and 12h00 hours and fixed for 24 hours in Carnoy's fixative. Anthers were then squashed in aceto-carmin (Darlington & La Cour, 1976). The multivalent configurations during diakinesis were emphasized for meiotic analysis.

RESULTS

The sample studied comprised 13 diploids, 12 triploids, 9 tetraploids, 3 pentaploids and 2 hexaploids (Table 1) and confirms our earlier report (Spies & Stirton, 1982) that *L. camara* in South Africa is a polyploid complex.

The early stages of meiosis were superficially similar in all the different cultivars although, during pachytene and diplotene, more chromosomes were visible in the higher polyploids (Fig. 1). Two chromosomes were always associated with the nucleolus at all polyploid levels.

Chromosome configurations during diakinesis ranged from univalents to heptavalents, but were predominantly bivalents (Fig. 2). The number of chiasmata during diakinesis, as well as the different configurations formed, are shown in Table 1. From Table 2 it can be deduced that there are big genome differences between different cultivars at the same polyploid level. These genome differences result in different chromosome associations.

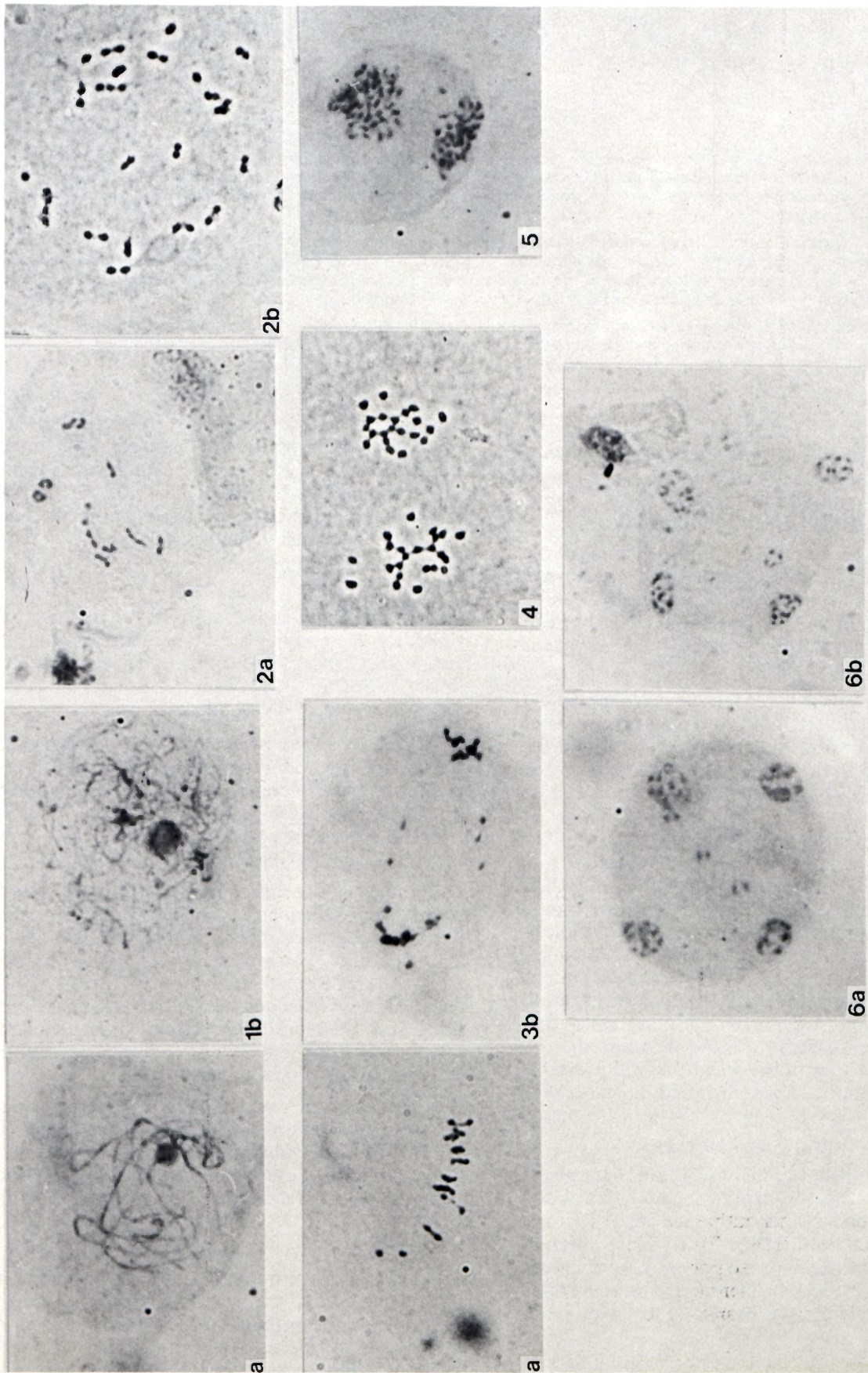
A high frequency of unpaired chromosomes and laggards (Fig. 3) were encountered during anaphase I (Table 3). Secondary association of chromosomes during telophase I usually occurred (Fig. 4). Neither heteromorphic bivalents nor B-chromosomes were found.

Instead of studying metaphase II and anaphase II, we concentrated our attention on the number of micronuclei found during telophase II (Table 4). The triploids and pentaploids usually deviated from the expected 4 nuclei. The size and number of the additional nuclei varied significantly (Fig. 6).

In *Stirton* 7387, a triploid plant, chromatid segregation in two cells was noticed instead of chromosome segregation. No bridges were obser-

* Botanical Research Institute, Department of Agriculture and Fisheries, Private Bag X101, Pretoria, 0001.

** New address: c/o Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, United Kingdom.



FIGS 1-6.—Meiosis in *Lantana camara*. 1, pachytene in a diploid ($2n=22$) (a), and a pentaploid ($2n=55$) (b) plant. 2, late diakinesis in a diploid (a), and tetraploid (b) plant. 3a, normal late metaphase I. 3b, anaphase I showing lagging chromosomes. 4, secondary association of chromosomes. 5, telophase I in a $2n=33$ plant; although chromosome counts are impossible, this is apparently a $33-33$ segregation. 6a & b, telophase II showing additional micronuclei of various sizes.

ved, but one fragment in one cell of a pentaploid cultivar (*Stirton* 7430) was recorded.

DISCUSSION

L. camara is a polyploid species with a basic chromosome number of 11 (Tandon & Chandi, 1955). The South African cultivars of this weedy species represent all the known polyploid levels (Spies & Stirton, 1982). Sharma & Mukhopadhyay (1963) quoted reports indicating aneuploidy in *L. camara*. The only aneuploid form deviating from a basic number of 11 is the $2n=32$ plant found by Schnack & Covas (1947). The *camera lucida* drawing presented by them, indicates that their plant could be a triploid ($2n=33$) with a 17-16 chromosome distribution in the late anaphase I illustrated.

The number of different chromosome configurations found increased with an increase in polyploid level. Significant variation within each polyploid level was found. Each polyploid level will now be discussed separately.

a) *The diploid group*

In the diploid group of 13 plants, one plant (*Stirton* 7066) showed bivalents only, whereas the other extremity (*Stirton* 7315) had 6,14% trivalents and 18,41% univalents, leaving 75,45% bivalents (Table 1). These differences clearly indicate a heterogeneous group with *Stirton* 6882, 7066, and 7374 being the only homogeneous diploid plants. Analysis of the bivalent configuration types indicated that *Stirton* 7066 and 7374 are more closely related to each other ($r=0,9791$) than they are related to *Stirton* 6882 ($r=0,8836$ and $0,9565$ respectively), because a higher correlation indicates a higher degree of similarity.

Multivalent association analysis of the associations presented in Table 2 indicates a high correlation between *Stirton* 7374, 6882, 7066, 7294, 7304, 7389, 7311 & 7361. In this group the homogeneous diploid plants (*Stirton* 6882, 7066 & 7374), or genetically similar plants, must have acted as some of the parental forms involved in hybridization during the formation of this group. Abnormal meiosis indicates that the rest of the group are hybrids. Morphological studies are required to indicate whether these three plants represent three different subspecies and also which related hybrids were formed by them.

The above-mentioned group is related to another smaller group consisting of *Stirton* 7306, 7339 & 7062. The latter group is characterized by equal frequencies containing configurations of 11_{11} and $10_{11} 2_1$, but with less $9_{11} 4_1$ configurations and with at least 17,7% of the cells containing configurations with one trivalent involved.

Stirton 7315 might be distantly related to *Stirton* 7306 ($r=0,5466$), whereas *Stirton* 7314 showed no correlation with and is, therefore, not related to any other diploid plant.

The diploid plants displayed little variation ($s=0,53$) with respect to the total number of chiasmata formed per genome. The chiasmata always occurred in the near terminal or terminal

parts of the chromosome. Table 1 shows that in 89,09% of the bivalents formed, only one chiasma was found. Of this number, 63,92% of the chiasmata were terminal. Notwithstanding this high figure of terminal associations, cases with two terminal chiasmata (4,01%) were much lower than cases with one terminal and one near terminal chiasma (6,88%).

All the trivalents encountered were of the chain type with two chiasmata. No correlation exists between the number of chiasmata found and the occurrence of trivalents.

During anaphase I regular chromosome segregation occurred in 93,85% of the cells studied (Table 3). In a single plant (*Stirton* 7294) less than 90% normal anaphases were encountered (60%). One cell had a 11-10-1 distribution, whereas three cells had two laggards in a 11-9-2 distribution. No explanation can be advanced as to why this plant with its relatively regular metaphase I exhibits such an irregular anaphase I.

The second meiotic division seems quite normal in this diploid group. Only two cells (1,54%) had an additional micro-nucleus during telophase II.

Published information describes bivalents only during metaphase I and a regular anaphase I with a high pollen fertility (Tandon & Bali, 1955; Tandon & Chandi, 1955; Natarajan & Ahuja, 1957). The only indication of abnormality was found by Khaleel & Nalini (1972) where a large number of pollen grains remained uninucleate. It is, therefore, clear that the South African diploid cultivars of *L. camara* differ greatly from their Indian counterparts as regards chromosomal behaviour.

The high average number of univalents (7,83%) found in South African diploid cultivars, indicates a high degree of incomplete homology between chromosomes, resulting in asynapsis. This might imply that most of the diploid plants encountered in this study, originated as hybrids between distantly related cultivars or possibly different subspecies of *L. camara*.

Trivalents were found in 2,02% of the chromosome associations of diploid plants. This figure is also too high to account for the occasional trivalent that may sometimes be found. Three causes for this high percentage of trivalents might exist. Either 11 is not the basic chromosome number or these diploid plants are actually allopolyploids derived from allotetraploids or minor genomic differences exist in this species resulting in abnormal hybrids. To determine which cause is responsible, the probability of occurrence of each one must be discussed.

If *L. camara* had originated as a hybrid between two related plants with two different basic chromosome numbers (e.g. 5 and 6), partial homology between the genomes must exist in some cases resulting in occasional trivalent formation. The expected quadrivalent formation was not encountered because of the low frequency of chiasmata and the relative low number of cells studied. No homology between the parental genomes would have resulted in normal 'diploids' (actually allotetraploids) which seems to be the case with *Stirton* 7066.

TABLE 4.—The number of micronuclei per cell during telophase II. (Percentage of cells within each class.)

Stirton No.	Normal (4 nuclei)	4 + 1	4 + 2	4 + 3	4 + 4	4 + 5	4 + 6
(a) Diploids							
6882	100						
7062	100						
7066	100						
7294	90	10					
7304	100						
7306	100						
7311	100						
7314	100						
7315	100						
7339	90	10					
7361	100						
7374	100						
7389	100						
(b) Triploids							
6878	10	50	20	20			
6883	5	35	45	5	5	5	
7067	50	40	10				
7348	10	30	50	10			
7381		20	30	40	10		
7382	10	30	30	20	10		
7383	10	20	10	30	20	10	
7384	20	50	30				
7387	20	30	40	10			
7393	20	30	30	10	10		
7394	10	20	10	40	10		10
7398		60	30	10			
(c) Tetraploids							
5288	100						
6784	100						
7060	100						
7087	100						
7230	100						
(d) Pentaploids							
5287	10	20	20	40	10		
7430	20	20	30	30			
7432	20	40	30	10			
(e) Hexaploids							
7058	80	20					
7431	90	10					

The frequency of trivalents indicates allopolyploidy with *Stirton* 7348 & 7394 tending towards autopolyploidy and *Stirton* 7381 & 7383 tending towards allopolyploidy. The remainder of the plants were distributed between these extremes.

The chromosome association pattern encountered during this study differs significantly from the results obtained by Natarajan & Ahuja (1957). About half their number of trivalents and twice their number of bivalents were found in this study. This clearly indicates that the Indian material differs quite considerably from the South African material. Where the Indian material might represent autotriploid plants, the South African plants are definitely segmental allopolyploids. Whereas the South African material suggests a basic chromosome number lower than 11 (more than 11 bivalents and trivalents are present), the Indian plants give no indication of a basic chromosome number lower than 11 (Natarajan & Ahuja, 1957).

A high frequency of univalents was encountered.

In addition to the univalents resulting from asynapsis, univalents are expected in triploid plants when bivalents are formed. The higher chromosome number produced more homologue chromosomes in the triploid compared with the diploid plants, so the role of asynapsis was diminished. The formation of quadrivalents is insignificant.

Very little variation ($s=0.26$) in the number of chiasmata formed was encountered. The number of terminal one chiasma bivalent configurations is lower in the triploid than in the diploid group. The number of terminal two chiasmata bivalents and terminal-subterminal two chiasmata bivalents is almost doubled. This led to a considerable increase in the number of chiasmata per genome compared to the diploids.

In addition to the chain type trivalents, a star type trivalent was encountered in 8.48% of the trivalents formed. This indicates that two chiasmata can occur close to each other, since this type of configuration requires a terminal chiasma between two chromo-

mes and a subterminal chiasma between one of them and another chromosome. All quadrivalents were of the chain type.

Anaphase I revealed vast differences between the triploid plants. In most of the cells studied a 18–15 (25%) or 17–16 (20,5%) distribution was found. One cell of *Stirton* 7384 acted as an allotriploid plant should, resulting in a 11-11-11 distribution. The other cells studied contradicted this observation.

In two cells of another plant (*Stirton* 7387) chromatid segregation occurred during anaphase I. If these cells had reached maturity, they would have had triploid pollen with the potential to increase the polyploid level of *L. camara* once again.

The abnormal anaphase I is reflected in an irregular second division. Only 30,08% telophase II cells had the expected 4 nuclei. As this does not guarantee that all the normal telophase II cells will develop into fertile pollen, the South African triploid *L. camara* will have less fertile pollen than their Indian counterparts (Tandon & Bali, 1955).

In both South African and Indian material an irregular meiosis was observed (Khoshoo & Mahal, 1967). Whereas the South African material mostly had a high number (more than 11) of bivalents, the Indian material had mostly $3_{111} 6_{11} 12_1$ (Tandon Bali, 1955) associations. The occurrence of more than 11 bivalents and trivalents in the South African material indicates homology between more than two genomes, therefore a basic chromosome number of less than 11 seems possible.

c) *The tetraploid group*

The tetraploid plants were a heterogeneous group (Table 1). The group had mostly bivalents, followed by trivalents, quadrivalents, univalents, a few pentavalents and even a hexavalent. Within the group large differences were encountered. The number of bivalents formed varied from 55,27% in *Stirton* 7230 to 82,8% in *Stirton* 7351. No evidence of an allotetraploid-like *L. camara* var. *mista* Bailey with only bivalents (reported by Natarajan & Ahuja, 1957) was found.

Whereas *Stirton* 6784, 7312 & 7351 had significantly more quadrivalents than trivalents, *Stirton* 7060, 7230, 7253 & 7270 had significantly less quadrivalents than trivalents. *Stirton* 5288 and 7087 were intermediate between these groups. Although this finding may appear to indicate three groups, the cytogenetical differences between the plants in each 'group' are too large to group them together.

During this study a much lower incidence of univalents and a higher number of bivalents was encountered than Natarajan & Ahuja (1957) reported.

The high frequency of multivalents indicates a segmental allopolyploid origin with an inclination towards autopolyploidy. Slightly more chiasmata per genome were encountered in the tetraploid group than in the triploids (Table 1). A very high frequency (72,64%) of terminal one chiasma bivalents were formed. The trivalents were mostly of the chain type with a significant increase in the number of the star types (23,12%). Five different

quadrivalent types were encountered. Usually the chain type (70,54%) and a ring of four (16,57%) were seen. The other types included a star (5,9%), a T (5,34%), and a terminal bivalent ring associated with a terminal bivalent chain (1,65%), to form a quadrivalent.

Pentavalents were encountered in one plant (*Stirton* 7230). The pentavalent consisted of a chain (33,333%) or of a ring of four with one chromosome associated in a near terminal chiasma with the ring. The hexavalent was in the form of a ring.

The few anaphase I cells suitable for analysis seemed to be normal. Telophase II was normal with four nuclei:

d) *The pentaploid group*

Unfortunately only three pentaploid plants were encountered during this study. Multivalent association analysis indicated that these plants are totally different. The differences are evident in every cytogenetic aspect examined.

One plant (*Stirton* 5287) had no pentavalents, and the other two plants had 7,73% and 6,82% pentavalents respectively. The number of univalents (6,21%) is much lower than expected after studying the triploid group (10,37%). The high chromosome number obtained by segmental allopolyploidy could have increased the number of homologue chromosomes to rule out asynapsis at the higher polyploid levels.

The total number of chiasmata per genome differs from 5,89 to 6,99. Bivalents were usually of the chain type with terminal chiasmata. Only *Stirton* 7430 had bivalents with two chiasmata. All trivalents were of the chain type. With the exception of *Stirton* 7432, quadrivalents were usually of the chain type with approximately a quarter of all quadrivalents of the star type. In *Stirton* 7432 half the quadrivalents were of the star type with an equal amount of chains and rings. Pentavalents were usually of the chain type, followed by a T-type and an elongated star type.

Anaphase I was observed in *Stirton* 7432 only and it seemed to be quite irregular with between 2 and 10 laggards. Irregular meioses in pentaploid *L. camara* plants were also described by Khoshoo & Mahal (1967). Telophase II revealed an irregular meiosis with 4 nuclei in only 16,7% of the cells studied.

e) *The hexaploid group*

Only two plants were found and studied. In almost all cytogenetical aspects these two plants differ. No correlation could be found by multivalent association analysis. The same number of multivalents are formed in each plant but the frequencies of the different multivalent configurations varies significantly. The figures obtained during this study correspond well with Natarajan & Ahuja's (1957) figures, except for the number of quadrivalents where twice their number was observed.

No terminal bivalent rings were found. Trivalents consisted usually of chains (83,34%) and stars (13,44%), but a terminal bivalent ring associated with a near terminal chiasma to another chromoso-

me was found in 6,45% of the trivalents. Quadrivalents occurred mainly as chains (56,43%) and stars (33,54%) with an occasional ring (10%). Pentavalents were found as chains (90,63%) and T's (9,38%). The hexavalents had chains (89,46%) and rings (11,54%) while all heptavalents were chains.

Anaphase I seemed normal and an extra micronucleus was encountered in only one telophase II cell (Table 4).

CONCLUSIONS

L. camara is a polyploid species. Since all polyploid forms encountered are multiples of 11 this number has been considered to be the basic chromosome number. The results of this study indicate either a lower basic chromosome number than 11, or postspeciation evolution of the genome must have occurred.

Cytogenetical differences at all polyploid levels indicate the occurrence of different genomic compositions in *L. camara*. This indicates either the introduction of different cultivars followed by hybridization in the past or a high rate of post introduction evolution has occurred within this species. At the diploid level at least four different groups exist with signs of hybridization.

The South African and Indian cultivars of *L. camara* reveal quite different chromosomal behaviour, implying that either different cultivars were introduced into these countries, or post introduction evolution in both countries resulted in different cultivar assemblages.

The polyploid forms of South African cultivars of *L. camara* are segmental allopolyploid plants.

UITTREKSEL

Lantana camara L. is 'n poliploïede spesie met 'n basiese chromosoomgetal van 11 ($x=11$). Die chromosoom assosiasies van 39 kultivars het aange-ton dat monovalente tot heptavalente voorkom met hoofsaaklik bivalente. Multivalent assosiasie analise toon aan dat minstens vier verskillende diploïede *L. camara* groepe in Suid-Afrika voorkom.

Geslagtelike voortplanting moet potensieel moontlik wees op diploïede vlak om verbastering moontlik

te maak, aangesien slegs verbastering die waargenome verskille in chromosoomgedrag kan verklaar. Dit is moontlik dat die basiese chromosoomgetal laer as 11 is of dat genoom ewolusie na spesie vorming plaasgevind het. Geen sitogenetiese korrelasie bestaan tussen die Suid-Afrikaanse en Indiese kultivars nie.

Die aantal chiasmata per genoom vermeerder namate die poliploïede vlak styg. Meeste multivalente is van die ketting tipe. Monovalente gedurende diakinese kan aan asinapsis toegeskryf word. Triploïede en pentaploïede plante toon abnormale meioses. *L. camara* is 'n segmentele allopoliploïed.

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