# TETRASOMATY IN THE ROOTS OF *CICER ARIETINUM* LINN.

By G. Meenakshi and M. K. Subramaniam

(*Cytogenetics Laboratory, Department of Biochemistry, Indian Institute of Science, Bangalore*–12)

Received October 13, 1967

## Abstract

Sporadic instances of tetrasomaty in the primary and secondary roots of five varieties of *Cicer arietinum* Linn. are illustrated. The phenomenon was more common in Varieties I and II. The SAT-chromosomes are reliable guides to estimate the degree of polysomaty, since pre-treatment with *p*-dichlorobenzene revealed that cell types with 32 chromosomes at metaphase had two pairs of SAT-chromosomes associated with the nucleolus at prophase. Tetrasomatic cells appear to be limited to the dermatogen and periblem. Higher degrees of polysomaty were not observed.

## Introduction

In *Allium cepa*, rare instances of haploidy and tetrasomaty were observed in successive crops of roots from the same bulb (Royan-Subramaniam, 1964; Subramanyam, 1964). In *Pisum sativum* rare anaphases had 49 (7*n*) and 21 (3*n*) chromosomes (Gopinath and Royan-Subramaniam, 1964). While in *A. cepa* the origin of the haploid and tetrasomatic cells could be surmised to be the consequence of spindle abnormalities in different directions, asynchronous replications of chromosomes have also to be visualized to explain the peculiar segregations in *Pisum sativum*. In the above context a detailed study of polysomaty in *Cicer arietinum* (Milovidov, 1932; Iyengar, 1939; Meenakshi and Subramaniam, 1963 *b*) was considered interesting.

In the cells of the root-tips of *Cicer arietinum*, the SAT-chromosomes are prominent in the side views of meta- and ana-phases (Meenakshi and Subramaniam, 1962, 1963 *a*). Using the SAT-chromosomes as markers it was possible to detect a rare triploid (Meenakshi and Subramaniam, 1966). A few of the cells in some of the roots were observed to have four SAT-chromosomes. The rarity of such cells and their presence near the actively dividing meristematic cells necessitated their consideration as a category distinct from the meristematic cells and concerned probably with tissue differentiation.

## Polysomaty

The occurrence of tetra-, octo- and 16-ploid cells in the tissues of plants whose meristematic cells had only a diploid chromosome complement was termed polysomaty by Langlet (1927). This phenomenon observed initially in *Spinacia* by Stomps (1910) appears to be common, especially in dicotyledons, and its origin is conceived to be by a double chromosomal reproduction in an otherwise normal mitotic cycle (Berger, 1941 *a*; Lorz, 1947; D'Amato, 1952; Royan and Subramaniam, 1954; Partanen, 1959; Bouharmont, 1960; Das, 1963). The polysomatic cells have been observed in the pariblem (cortical ground meristem), dermatogen (protoderm) and in rare instances in the plerome (procambium) as well. From the size of the nucleoli and the number of heterochromatic satellites in association with them, Berger (1941 *b*) has shown that polysomaty occurs in the periblem of the radicle of *Spinacia* even before the beginning of germination. In leguminous plants, polysomatic cells were found at a definite time and place during development (Berger, Witkus and McMahon, 1958). The presence or absence of polyploid divisions can even be used as a criterion in studies on the systematics of Leguminosae (Berger, Witkus and McMahon, 1958).

## Material and Methods

Four varieties which could be distinguished on the basis of the size and colour of their seeds were used for this study. These were: Variety I with big white seeds; Variety II with small white seeds; Variety III with fairly big green seeds and Variety IV with small brown seeds. Another commercial strain of *C. arietinum* purchased at the local market was also investigated.

Primary and secondary roots of the five varieties were fixed in acetic alcohol (1 : 3) for aceto-carmine, haematoxylin and Feulgen squashes and in Navashin's and Lewitsky's (1% chromic acid and 10% formalin 1 : 1) fluids for staining serial longitudinal and transverse sections with haematoxylin as well as Feulgen.

Some of the material was exposed to a saturated solution of *p*-dichlorobenzene for 90 minutes prior to fixation in acetic alcohol to enable an accurate estimate of the chromosome number and evaluate the relation between the SAT-chromosomes and nucleoli in squashes (Meenakshi and Subramaniam, 1963 *b*).

## Observations

The criteria employed for the identification of polysomaty were: (1) the size of the nuclei and the number of chromocenters in them ; (2) the chromo-

some number of such cells; (3) the number of SAT-chromosomes in the complement and (4) the number of SAT-chromosomes associated with the nucleolus.

To locate and study the distribution of polysomatic cells in the meristematic layers, sections had to be scanned. But the inherent limitations of sections preclude the use of all the criteria enumerated above.

In squashes, the cell and nuclear sizes are unreliable and hence more reliance had to be placed on the number of chromocenters. To estimate the chromosome number in squashes, pre-treatment of the material with an agency which inactivates the spindle became essential. Therefore, in the descriptions that follow, the observations are collated from the different methods employed.

*Sections*

The polysomatic cells were confined to the dermatogen (protoderm) and to the 2 or 3 outer layers of cells of the periblem (cortical ground meristem). The first identification of polysomaty was in one of the fifty roots of the commercial variety examined. Though such cells were observed more frequently in Varieties I and II, the easy germination of the former led to a concentration of attention on this strain alone. No evidence for polysomaty was obtained in 12 of the primary roots and in only 3 among the 30 secondary roots were such cells observed.

*Squashes*

In squashes, the polysomatic cells occurred singly or in groups of 2 or more near the diploid ones (Photo 1). Their occurrence in Varieties I and II was about 2–5%. In two instances they constituted almost 10% of the cell population. They were less than 2% in Varieties III and IV, though such cells were relatively more in Variety III.

*Untreated Roots*

*Interphase and Prophase.*—Tetrasomatic interphase and prophase nuclei are shown in photos 2 and 3 respectively. The number of chromocenters in photo 2 exceeds sixteen. In the tetrasomatic prophase shown in photo 3 the satellites are seen on the nucleolus, but their mode of attachment is not clear. In a rare prophase (Photo 4) the chromosomes occurred as pairs. Only 15 such pairs could be distinguished clearly.

*Metaphase.*—Metaphases from the diploid (Photo 5) and polysomatic (photos 6 and 7) cells from haematoxylin (Photos 5 and 6 of Variety II) and Feulgen-aceto-carmine (Photo 7 of Variety I) squashes are presented for comparison. The metaphases exhibited in Photos 6 and 7 have twice the number of chromosomes. The pair of SAT-chromosomes is clear in the diploid (Photo 5) while only three SAT-chromosomes could be located clearly in the tetrasomatic metaphases (Photos 6 and 7). The two examples illustrated in Photos 5 and 6 were from the root of the exceptional seed of Variety II which had normal instead of tandem satellites (unpublished data).

*Anaphases.*—In side views of anaphases, the overlapping of the chromosomes necessitated the use of satellites as guides to estimate the degree of polysomaty. A diploid anaphase with a pair of tandem satellites in each group is illustrated in Photo 8. The two pairs of SAT-chromosomes in the top group of the anaphase shown in Photo 9 implies that each daughter anaphase group has thirty-two chromosomes.

*Roots treated with p-dichlorobenzene*

The contraction of the chromosomes produced by *p*-dichlorobenzene enabled the identification of the satellites at prophase. In Photo 10 the sixteen chromosomes are easy to count. The intercalary SAT-grain is connected to the arm of chromosome A (Photo 10) by two SAT-threads while the SAT-thread of chromosome B is rather hazy. Two pairs of tandem satellites could be identified in Photo 11 of a tetrasomatic cell. The threads connecting the SAT-grains to the chromosome body are, however, not visible.

Unlike in the diploid where only a pair of SAT-chromosomes is associated with the nucleolus at prophase (Meenakshi and Subramaniam, 1963 *a* and *b*), four SAT-shromosomes could be demonstrated in association with the nucleoli in tetrasomatic prophases. In Photo 12, four chromosomes with tandem satellites (A, B, C and D) are shown in intimate association with the nucleolus. Two SAT-threads connect the intercalary grain to the body of chromosome A, while the pair of threads could be traced from the intercalary to the terminal grain in chromosome C. In chromosome D, the SAT-thread between the chromosome body and the intercalary grain is enclosed in the nucleolar matter and a relatively deeply stained ring (*cf.* Photo 2 of Meenakshi and Subramaniam, 1963 *b*) could be made out (Photos 13 and 13 A).

The chromosomes shown in Photo 14 occur as 16 discrete pairs. *p*-dichlorobenzene is said to keep the chromosomes at metaphase only for a

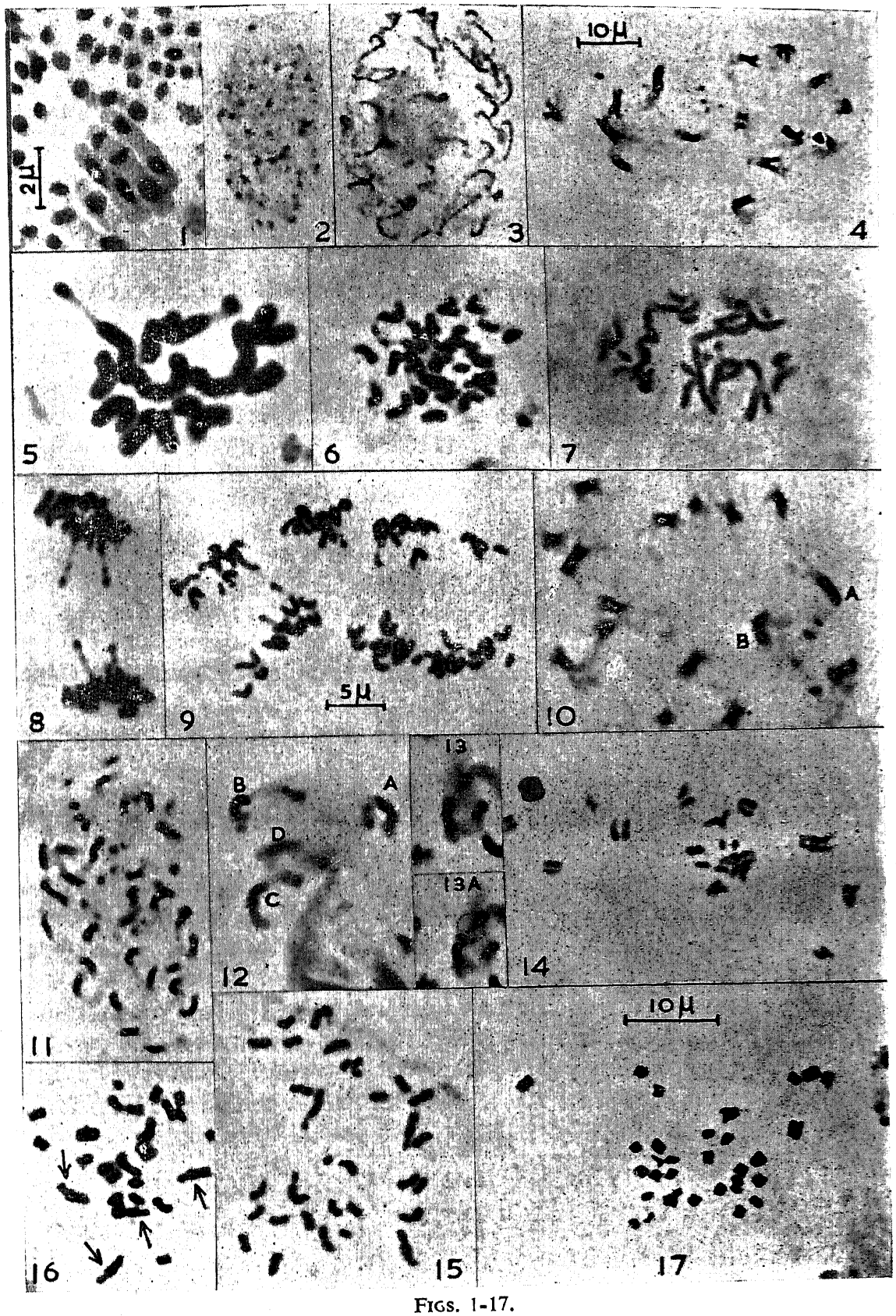
limited duration (Evans and Tonkinson, 1959). Other diploid anaphases have also been observed in treated material. The thirty-two diploid anaphase chromsomes appear scattered in Photo 15. In the above context it became necessary to separate the anaphase of a diploid from the metaphase of a tetrasomatic cell. This is possible from (*a*) the width of the metaphase chromosomes and (*b*) the presence of a longitudinal split in many of them (Gopinath and Royan-Subramaniam, 1964). Besides, the anaphase chromosomes had a tendency to accumulate a lighter staining substance around them (Meenakshi and Subramaniam, 1963 *b*).

Photos 16 and 17 are of metaphases of tetrasomatic cells. The action of *p*-dichlorobenzene being not uniform, the contraction induced is often to different degrees. While the four SAT-chromosomes (indicated by arrows) are clear in Photo 16, their morphology becomes indistinct when the chromosomes are highly contracted (Photo 17). The longitudinal split is distinct when they are highly contracted and thirty-two chromosomes could be counted clearly in Photo 17.

## DISCUSSION

The occurrence of polysomaty appears to be sporadic in the roots of *C. arietinum* as reported by Iyengar (1939). Whether it is a regular phenomenon during tissue differentiation can only be judged by stimulating the differentiated cells to divide. The fact that some of the polysomatic cells were in division enabled the use of several criteria. While sections are necessary to locate the position of the cells, an accurate estimate of the chromosome number is rendered possible in squashes by the use of C-mitotic agents (Berger and Witkus, 1943; Berger, McMahon and Witkus, 1961).

A further advance is the use of heterochromatic regions of satellites associated with the nucleoli to judge the degree of polyploidy in resting nuclei (Berger, 1941 *a* and *b*). These have been extended in the present investigation by the use of haematoxylin as a stain for squashes and a critical study of the number and mode of association of the satellites to the nucleolus at prophase in roots treated with *p*-dichlorobenzene (Photos 11 and 12). This was a logical extension of the analysis of the SAT-nucleolus relationship in normal diploid prophases (unpublished data). It could be shown in favourable instances of tetrasomatic prophases, that the SAT-threads and grains are often double (A and C in Photo 12) (Royan-Subramaniam and Subramaniam, 1965), that the nucleolar matter surrounds the SAT-threads (Patau, 1937; Pathak, 1940; Mulnard, 1956; Subramanyam and Royan, 1962; Meenakshi




FIGS. 1-17.

and Subramaniam, 1963 *a*; Gopinath and Subramaniam, 1963), and that in such regions a deeply stained ring could sometimes be discerned (Photos 13 and 13 A) (Meenakshi and Subramaniam, 1963 *b*).

The occurrence of chromosomes in pairs in polysomatic cells (Berger, 1941 *a*; Berger, Mc Mahon and Witkus, 1961) was seen only in a single prophase (Photo 4) and hence does not appear to be a common feature in *C. arietinum*. A critical distinction of a diploid anaphase from a tetrasomatic metaphase is also possible (Gopinath and Royan-Subramaniam, 1964) in pre-treated material by the presence of a longitudinal split only in the metaphase chromosomes (Photos 16 and 17). It would appear that chromosome numbers higher than tetrasomaty do not occur in *C. arietinum*.

## References


Berger, C. A. .. "Reinvestigation of Polysomaty in *Spinaca*," *Bot. Gaz.*, 1941 *a*, **102**, 759–69.

———— .. "Some criteria for judging the degree of polyploidy of cells in the resting stage," *Amer. Nat.*, 1941 *b*, **75**, 93–96.

———— and Witkus, E. R. "A cytological study of C-mitosis in the polysomatic plant, *Spinacia oleracea*, with comparative observations on *Allium cepa*," *Bull. Torrey Bot. Club.*, 1943, **70**, 457–67.

——, Mc Mahon, J. and Witkus, E. R. "Naturally occurring polyploidy in the development of some species of *Allium*," *Caryologia*, 1961, **14**, 383–89.

——, Witkus, E. R. and Mc Mahon, R. M. "Cytotaxonomic studies in the Leguminosae," *Bull. Torrey Bot. Club*, 1958, **85**, 405–15.

Bouharmont, J. .. "Mitose et Polysomatie chez *Mimosa invisa* Mart", *La Cellule*, 1960, **61**, 7–17.

D'Amato, F. .. "Polyploidy in the differentiation and function of tissues and cells in plants," *Caryologia*, 1952, **4**, 311–58.

Das, B. C. .. "Polysomaty in Mulberry," *Sci. & Cult.*, 1963, **29**, 250–52.

Evans, H. J. and Tonkinson, S. M. "A paradoxical effect of aeration on the accumulation of root-tip metaphase cells by spindle inhibitors," *Exptl. Cell Res.*, 1959, **17**, 262–71.

Gopinath, P. M. and Royan-Subramaniam, S.. "Peculiar chromosomal groupings in the meristematic and polysomatic cells of *Pisum sativum* L.", *Caryologia*, 1964, **17**, 465–70.

—— and Subramaniam, M. K. "Persisting nucleoli and micronuclei in the mitotic cells of *Pisum sativum* Linn.," *Curr. Sci.*, 1963, **32**, 169–71.

Iyengar, N. K. .. "Cytological investigations on the genus *Cicer*," *Ann. Bot., N.S.*, 1939, **3**, 271–305.

*Langlet, O. .. "Zur kentniss der polysomatischen Zellkerne in Wurzelmeristem," *Svensk. Bot. Tidskr.*, 1927, **21**, 169–84.

Lorz, A. P. .. "Supernumerary chromonemal reproductions: Polytene chromosomes, Endomitosis, Multiple Chromosome Complexes, Polysomaty," *Bot. Rev.*, 1947, **13**, 597-624.

Meenakshi, G. and Subramaniam, M. K. "Variations in the satellited chromosomes of *Cicer arietinum* Linn.," *Proc. Ind. Acad. Sci.*, 1962, **55B**, 15–23.

———— .. "Studies on the Nucleolus. II. The Persisting Nucleoli of *Cicer arietinum* Linn.," *Ibid.*, 1963 *a*, **57B**, 73–82.

———— .. "Nucleolus-Satellite relationship at prophase and Polysomaty in *Cicer arietinum* Linn.," *Curr. Sci.*, 1963 *b*, **32**, 465–67.

———— .. "Triploidy in *Cicer arietinum* Linn.", *Caryologia*, 1966, **19**, 163–166.

*Milovidov, P. F. .. "Pripad mixoploidie u *Cicer arietinum* Linn. Preslia," *Véstink Ceskoslovenski Botanicki Spolecnosti V Praze* 1932, **11**, 1–5.

*Mulnard, J. .. "Presence d'inclusions Feulgen-positives dans les nucleoles larvaires de quelques Dipteres," *Arch. Biol.*, 1956, **67**, 485–98.

Partanen, C. R. .. "Quantitative chromosomal changes and differentiation in plants," In *Developmental Cytology*, D. Rudnick Ed., Ronald Press, New York, 1959.

Pätau, K. .. "SAT-Chromosome und Spiralstruktur der Chromosomen der extrakäpsularen Körper (Merodinium spec.). Von Collozoum inerme Müller," *Cytologia*, 1939, 667–80.

Pathak, G. N. ... "Studies in the Cytology of *Crocus*," *Ann. Bot., N.S.*, 1940, **4**, 227–56.

Royan, S. and Subramaniam, M. K. "A dynamic approach to tissue differentiation," *Curr. Sci.*, 1954, **23**, 315–19.

Royan-Subramaniam, S. .. "A rare haploid cell in a root of *Allium cepa*," *Ibid.*, 1964, **33**, 203–05.

———— and Subramaniam, M. K. "The structure of the Satellite Thread region of the chromosomes of *Allium cepa*," *La Cellule*, 1965, **65**, 193–98.

*Stomps, T. J. .. "Kernteilung und Synapsis bei *Spinacia oleracea*," *Biol. Centralbl.*, 1910, **31**, 257–309.

Subramanyam, S. .. "The structure of the diplochromosomes and a rare haploid cell in the roots of *Allium cepa*," *Curr. Sci.*, 1964, **33**, 217–19.

—— and Royan, S. .. "The persisting nucleolus of *Allium cepa*," *Ibid.*, 1962, **31**, 244–46.

Witkus, E. R. and Berger, C. A. "Polyploid mitosis in the normal development of *Mimosa pudica*," *Bull. Torrey Bot. Club*, 1947, **74**, 279–82.

* Not consulted in original.

## EXPLANATION OF PLATE I

Squashes of Varieties I and II. Acetic alcohol fixation.

Photos 10–17. From roots pre-treated with *p*-dichlorobenzene prior to fixation.

PHOTOS 1–6 AND 8–17. Haematoxylin. Photo 7. Feulgen-acetocarmine.

PHOTO 1. Illustrates the distribution of 4 *n* cells near the diploid ones.

PHOTOS 2 AND 3. Interphase nucleus with chromocentres and Prophase from tetrasomatic cells respectively.

PHOTO 4. Prophase (4*n*). Only 15 pairs of chromosomes are clear.

PHOTOS 5–7. Metaphases from diploid (Photo 5) and tetrasomatic cells (Photos 6 and 7). The pair of SAT-chromosomes is clear in Photo 5. Only three SAT-chromosomes are seen in Photos 6 and 7.

PHOTOS 8 AND 9. Anaphases from a diploid with a pair of SAT-chromosomes (Photo 8) and a tetrasomatic cell with 2 pairs of SAT-chromosomes (photo 9).

PHOTO 10. Diploid prophase with 16 chromosomes including a pair of SAT-chromosomes (A & B).

PHOTO 11. Prophase (4*n*). Four tandem satellites are seen in the middle of the cell.

PHOTOS 12–13 A. Prophase (4*n*). Four chromosomes (A, B, C & D) with tandem satellites are attached to the nucleolus (Photo 12). The deeply stained ring seen around the SAT-thread of chromosome D (Photo 13) is stippled with India ink in Photo 13 A.

PHOTOS 14 AND 15. Anaphases (2*n*). The chromosomes are lying in pairs in Photo 14. They are scattered in Photo 15.

PHOTOS 16 AND 17. Metaphases (4*n*). The four SAT-chromosomes are indicated by arrows in Photo 16. The 32 chromosomes illustrated in Photo 17 are highly contracted obscuring the satellites completely.

Magnification of Photos 2, 3 and 4 as given in Photo 4.
do. 5–10, 12–13 A and 16 as given in Photo 9.
do. 11, 14, 15 and 17 as given in Photo 17.