

## HETEROCHROMATIN DISTRIBUTION PATTERN IN FIVE VARIETIES OF *TRICHOSANTHES ANGUINA* L. FROM BANGLADESH

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### Abstract

Five commercial varieties of *Trichosanthes anguina* were studied cytogenetically for authentic characterization based on heterochromatic distribution pattern. The interphase nuclei of the variety Apurbo showed diffuse type of interphase nuclei, staining with homogenous distribution of chromatin in orcein staining. Complex chromocenter type with big heteropycnotic blocks were observed in the interphase nuclei of the rest four varieties namely, Turag, Dhaka green, Super long green and Anika. The prophase chromosomes of the variety Turag, Anika and Apurbo were homogeneously stained whereas these gradually stained in Dhaka green variety. The interstitial region of prophase chromosomes of Super long green were stained in orcein. These five varieties displayed distinction in GC-rich repeats distribution in interphase and prophase stage of mitotic cell division. Therefore, with the help of cytogenetical analysis, it was possible to characterize each variety based on its heterochromatin distribution pattern by differential staining.

### Introduction

Snake gourd (*Trichosanthes anguina* L.) is an important summer vegetable in Bangladesh belonging to Cucurbitaceae which has moderately high nutritive value and rich in minerals and vitamins. It grows throughout the year except in extreme winter. In addition, plants of this species have some medicinal and ethno-botanical importance. In spite of a diverse wide polymorphism in respect of fruit color, size and weight the total production of this species was low (Khatun *et al.* 2010). One of the causes of this crop's poor yield in Bangladesh is the absence of high-yielding varieties.

In Bangladesh, a number of seed companies such as Lal Teer, Bikrampur Seed Co., Green Agro Tech Service, Samrat Seed Company have been released different varieties of Snake Gourd with various trade names like Turag, Dhaka green, Super long green, Anika and Apurbo. All the companies claimed that the genetic purity of each variety is over 98% all though having no idea about the genetic makeup of the variety. For this reason, it is necessary to characterize authentically each variety of Snake gourd. There were some reports on their somatic chromosome number as well as molecular level cytogenetical analysis (Sarker *et al.* 1987, Alam *et al.* 2012, Alam *et al.* 2018, Bhowmick and Jha 2022).

In addition, the study of the heterochromatin distribution of interphase nuclei and prophase chromosomes by orcein and CMA is very useful to know the nature of heterochromatin and also get an idea about its distribution pattern (Sultana and Alam 2016, Afroz and Sultana 2022). Tanaka (1971) classified the different types of interphase nuclei and prophase chromosomes based on orcein staining properties. In this study, along with a thorough karyomorphological analysis, for the purpose of differentiating them based on their staining characteristics of prophase chromosomes and interphase nuclei has been done for *Trichosanthes anguina*. The outcome of this study showed that various taxa including varieties of many plant species could be distinguished by their staining properties.

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### Materials and Method

The seeds of five commercial *Trichosanthes anguina* varieties were collected from various seed suppliers, namely Turag and Dhaka from Lal Teer Seed Limited, Super long green from Bikrampur Seed Co., Anika from Green Agro Tech Services and Apurbo from Samrat Seed Company. The different seeds were germinated and grown in the botanical garden of the Department of Botany, University of Dhaka.

To know the heterochromatin distribution pattern with differential staining, Alam *et al.* (2012) method was followed in this study.

### Results and Discussion

With orcein staining, several darkly stained chromocenters were found in the interphase nuclei of the variety Turag. In some cases, fine threads like structures were also observed. No nucleolus or non-staining area was found (Fig. 1F). In the variety Dhaka green, 10-12 darkly stained more or less round shaped chromocenters were observed. The size of chromocenters was bigger than those of variety Turag. A large, noticeable nucleolus took up around one-third of the nucleus. A distinct non-staining region was present around the nucleolus (Fig. 1G). The interphase nuclei of the variety Super long green were found to possess several big and darkly stained regions. The chromocenters were aggregated and formed big heteropycnotic blocks. The rest of the nucleus was more or less stained well. No nucleolus was found (Fig. 1H). Many big and darkly stained heteropycnotic blocks were found in the interphase nuclei of the variety Anika. These blocks were the biggest among the five varieties studied. A large nucleolus occupied about 1/4<sup>th</sup> of the nucleus was observed (Fig. 1I). In the variety Apurbo, no prominent chromocenters or heteropycnotic block was found. The nucleus stained more or less homogeneously. A conspicuous nucleolus about 1/5<sup>th</sup> of the nucleus was observed (Fig. 1J).

The five types of snake gourd had varying interphase nuclei staining characteristics. Turag, Dhaka green, Super long green and Anika varieties had darkly stained chromocenters (Figs 1F-1I). According to the classification proposed by Tanaka (1971) this type of staining known as Complex Chromocenter Type (Table 1). On the other hand, no darkly stained chromocenter were found in the variety Apurbo rather the nucleus stained homogeneously (Fig. 1J). Tanaka described this type of interphase nuclei staining as Diffuse Type (Table 1). Based on staining property, the variety Apurbo is completely different from the others.

Big and prominent nucleoli were found in the variety Dhaka green, Anika and Apurbo (Figs 1G, 1I and 1J). The prominent appearance of nucleoli suggested the active transcription of rDNA that is late transcriptional as well. The presence of nucleolus reveals that ribosomal genes scattered among chromosomes. These three varieties differed from the other two in these features (Figs 1F-1J).

With GC-specific staining, several round bright bands were observed in the interphase nucleus in the variety Turag. The rest of the nucleus was stained well (Fig. 1K). The variety Dhaka green possessed one bright, prominent, and big CMA-band along with 3-4 less fluoresced big bands in the interphase nuclei (Fig. 1L). In the interphase nuclei of variety Super long green, 4-5 CMA bands were present of which one is comparatively larger and brighter than the other one. A non-staining region was observed in the nucleus. The CMA bands were arranged at the periphery of the non-staining region (Fig. 1M). Four to five big CMA fluoresced regions were found in the interphase nucleus of the variety Anika. These bands were not brightly stained (Fig. 1N). In the variety Apurbo, two conspicuous bright CMA bands were found in the interphase nuclei. These bands were seen in the parts of the nucleus that did not stain. In some cells, 1-2 min CMA bands were also observed ((Fig. 1O)

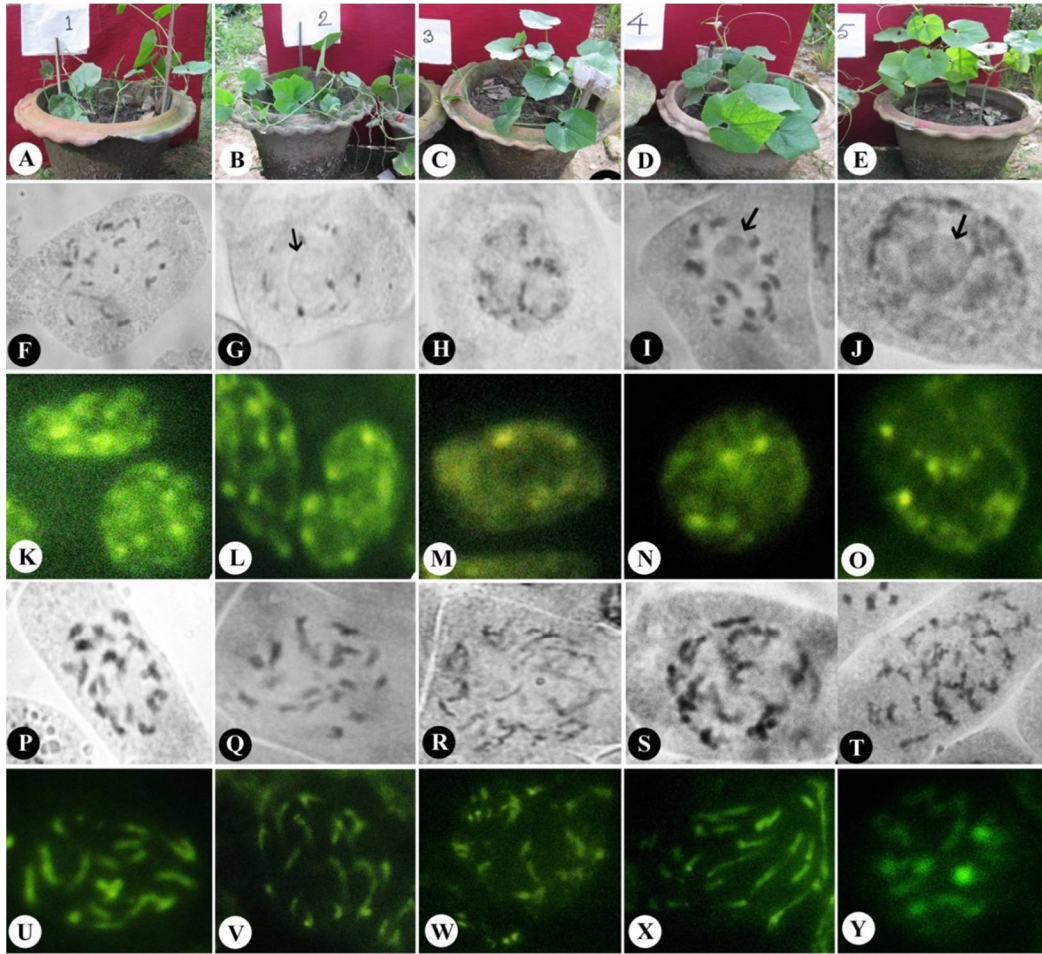


Fig. 1. Plant materials, Orcein, and CMA- stained interphase nuclei and prophase chromosomes of five *Trichosanthes anguina* varieties. Plant materials, Turag (A), Dhaka green (B), Super long green (C), Anika (D) and Apurba (E); Orcein stained mitotic interphase nuclei of Turag (F), Dhaka green (G), Super long green (H), Anika (I) and Apurba (J); CMA-stained mitotic interphase nuclei of Turag (K), Dhaka green (L), Super long green (M), Anika (N) and Apurba (O); Orcein stained mitotic prophase chromosomes of Turag (P), Dhaka green (Q), Super long green (R), Anika (S) and Apurba (T); CMA-stained mitotic prophase chromosomes of Turag (U), Dhaka green (V), Super long green (W), Anika (X) and Apurba (Y). Arrow indicates nucleolus. Bar = 10  $\mu$ m.

With orcein staining, the prophase chromosomes of the variety Turag, Anika and Apurbo more or less homogenously stained chromosomes were observed (Figs 1P, 1S and 1T). The prophase chromosomes in the variety Dhaka green stained gradually from one end to another (Fig. 1Q). Each chromosome had one end that was significantly darker than the other. In the variety Super long green, the prophase chromosomes were not stained properly. However, few darkly stained small regions were scattered in the chromosomal region (Fig. 1R).

The prophase chromosomes of the variety Turag, Anika and Apurbo stained homogeneously. Tanaka (1971) described this as Continuous Type of staining. In the Dhaka green variety, the prophase chromosomes were gradually darker from one end to another. Tanaka termed this as Gradient Type. Except a few dark regions, the prophase chromosomes of Super long green were

not stained. According to Tanaka (1971), it was classified as Interstitial Type of prophase chromosome. Thus, the varieties had their features of the staining properties of prophase chromosomes (Table 1).

**Table 1. Types of interphase nuclei and prophase chromosomes of *Trichosanthes anguina* L.**

Varieties	Type of orcein-stained interphase nuclei	Type of orcein-stained prophase chromosomes
Turag	Complex chromocenter	Continuous
Dhaka green	Complex chromocenter	Gradient
Super long green	Complex chromocenter	Interstitial
Anika	Complex chromocenter	Continuous
Apurbo	Diffuse	Continuous

After staining with GC-specific stain (CMA) at prophase stage, no banded region was found in the variety Turag. The chromosomes were homogeneously stained along the entire chromosomal length (Fig. 1U). In Dhaka green variety, brightly fluoresced CMA regions were found at the terminal end of few chromosomes (Fig. 1V). Four prominent CMA fluoresced regions were observed in the prophase chromosome of the variety Super long green. In addition, two less prominent fluoresced regions were also observed (Fig. 1W). In the variety Anika, the prophase chromosome had several CMA fluoresced regions. A number of the prophase chromosomes of this combination were fluoresced well in spite of the fact that the other showed up obscured district (Fig. 1X). Two big, bright and condensed CMA<sup>+ve</sup> regions were found in the prophase chromosome in the variety Apurbo (Fig. 1Y).

The characteristics of the mitotic interphase and prophase chromosomes of *Trichosanthes anguina* owing to differential staining with orcein and CMA were not reported in the literature or online resources that are currently available. Thus, the aforementioned criteria were used in a pioneering attempt to characterize five commercial varieties of *T. anguina*.

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