Study of nucleolus through hematoxylin staining and determination of nucleolar frequency

Principle:

The nucleolus is the most prominent membrane-less domain in the interphase nucleus. It has high density and greater refractive index relative to the surrounding nucleoplasm rendering it readily detectable in cytological preparations by light microscopy. This is a temporary part that developed at the secondary constriction region (NOR region) and appears during interphase and disappears during late prophase. Primarily it participates in assembly of the ribosomes and functions in rRNA synthesis. The nucleoli start to appear in very early interphase and number of nucleoli depends on the number of nucleolar chromosomes. It must be at least 2 and /or more per nuclear genome. In the late interphase, all the nucleoli fuse together and form a large prominent one.

The method use for nucleolus staining in aqueous haematoxylin stain was employed by Dey and Ghosh in 1987. The technique involves prolonged hydrolysis of root tip in higher temperature with 45% acetic acid. This results in denaturation of basic proteins. The structure like nucleolus which is rich in acidic proteins (where aspartic acid and butyric acid are the prevalent amino acids), when stain with haematoxylin shows differential stain. The nucleolus appears as a distinct body against a background.

Material:

Root tips of *Allium cepa*.

Reagents required:

1. Lily's Fixative; abbreviated as FAA [formaldehyde (formalin) : acetic acid : absolute alcohol :: 10 : 5 : 85]

2. 45% acetic acid

3. Freshly prepared 4% aqueous solution of iron alum

4. 0.5% aqueous hematoxylin solution

Procedure:

Fixation: Root tips of about 5mm are fixed in Lily's fixative overnight and kept under refrigeration (8-10^{o}C).

Hydrolysis: After washing thoroughly in distilled water, root tips are hydrolysed in 45% acetic acid at 82-85^{0}C in a water bath for 30 minutes.

Staining: The root tips are then washed in distilled water again and a few drops of freshly prepared saturated aqueous solution of iron alum are added. The iron alum acts as a mordant to increase the stainability. After 10-12 minutes root tips are directly transferred to 0.5% aqueous haematoxylin solution for 20-30 minutes in room temperature.

This is followed by squash in 45% acetic acid on a grease free slide and observed under high power of compound microscope.

Observation:

Under high power (45X) objective lens, the nucleolus looks like a large dark brown to bluish black stained spot within the nucleus. Each nucleus contains one or two or three nucleolus.

Result:

No. of observation	Total no. of cells in the microscopic field	Cells with 1 nucleolus	Cells with 2 nucleolus	Cells with 3 nucleolus	Total no. of nucleolus
1	24	13	11	0	35
2	20	11	07	2	31
3	19	07	12	0	31
4	22	18	06	0	30
5	21	09	11	1	34
	∑=106				∑=161

$$\text{Nucleolar frequency} = \frac{\text{Total number of nucleoli}}{\text{Total number of cells}}$$

$$= \frac{161}{106}$$

$$= 1.51$$

Conclusion:

Hence the nucleolar frequency in *Allium cepa* is 1.51 with 1 nucleolus predominating.