Cytological Investigations of Cowpea (*Vigna unguiculata* (L.) Walp) and Sem (*Lablab purpureus* (L.) Sweet) Two Major Fodder Legumes

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Cytological investigations of Cowpea (*Vigna unguiculata* (L.) Walp) and Sem (*Lablab purpureus* (L.) Sweet) two major fodder legumes

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Introduction

Indian subcontinent is one of the world's mega-centres for crop plant diversity as it has a wide spectrum of eco-climate ranging from humid tropical to semi-arid, temperate to alpine. The Indian gene centre possesses rich genetic diversity for forage legume genera as well. Germplasm characterisation and documentation is a major activity of conserving genetic resources, in this direction germplasm accessions were characterized for morphological and agronomic traits in batches over the years. The relative basic information on cytological characterization of two major forage legume genera Cowpea (*Vigna unguiculata* (L.) Walp) and Sem (*Lablab purpureus* (L.) Sweet) is not quite sufficient (Adetula, 2006; Bandyopadhyay *et al*., 2009). Therefore, present investigation was proposed to collect this information for few accessions of these two major fodder legumes. This information on diversity can be used in conservation strategies *i.e* maintenance of purity as well as in genetic breeding programs of the species.

Both gerera with Chromosome number 2n = 22 are predominantly self- fertilizing. All the cultivated cowpea, *Vigna unguiculata* (L.) Walp. is grouped under *Vigna unguiculata,* subdivided into four semi-groups or cultigroups *viz. unguiculata, biflora, sesquipedalis,* and *textilis*. Although most domesticated material of Sem is either ssp. *purpureus* or ssp. *bengalensis*, however, ssp. *uncinatus* of Sem has been domesticated in Ethiopia. Cytological investigation was performed for the studies of chromosome number, pairing behaviour and chromosomal association in case of both Cowpea and Semaccessions.

Materials and Methods

Meiotic chromosomes were studied from pollen mother cells using, Rapid Squash technique (Dyer, 1963). The flower buds were collected from net house of crop improvement division, IGFRI Jhansi, in cowpea in Kharif 2009 and of lablab in kharif 2013 in the morning time between 6.45 a.m. and 7.30 am in Carnoy's fixative solution having 3:1 ratio of absolute ethyl alcohol: acetic acid for carrying them to laboratory. The anthers from appropriate size of buds were smeared in Lacto propionic orcein and visualized in photographic compound microscope.

The metaphase plate, anaphase and diakinesis stages were observed in four accessions of Cowpea *V. unguiculata* including three culti groups i.e. *unguiculata* (2 accessions; IL-1177 and EC548999), *sesquipedalis* (1 accession; EC548875) and *cylindrical* (1 accession: IC 438864). The Pollen mother cells were observed for the study of meiosis in *Lablab purpureus* genotype Bundel sem-1.

Results and Discussion

The identification of individual chromosomes in case of these fodder legumes is generally limited by the extremely small size of the chromosomes, however, the types of associations were observed by the meiotic studies carried out in Pollen mother cells (PMCs) of three cultigroups of Cowpea *i.e. V. unguiculata* cv. gr. *unguiculata* (accessions, EC548999 and IL-1177), *V. unguiculata* cv. gr. *sesquipedalis* (accession, EC 548875) and *V. unguiculata* cv. gr. *cylindrical* (accession, IC 438864). Chromosome associations were noted during diakinesis, metaphase and anaphase in these accessions. All the accessions showed a uniform chromosome number of 2n=22 .Meiotic studies revealed the details of chromosome associations which varied from cell to cell both between and within different accessions. Five different types of associations in *V. unguiculata* cv. gr. *sesquipedalis* cultigroup accession, only four types of associations in cultigroup *V. unguiculata* cv. gr. *cylindrical* accessions were observed. Six types of associations were observed in *unguiculata* cultigroup accession with arrow shaped leaves and four types observed in *unguiculata* cultigroup accession with normal leaves.

The pooled data including 60 cells involving 1320 chromosomes showed that maximum *i.e*. 25% cells were associated as 11 bivalents followed by 9II + 4I and 1IV + 8II + 2I associations which were visualized in 20% cells each,

respectively. Chromosomal associations 1IV + 7II + 4I and 10 II+ 2 I was observed in 15% and 16.66% cells respectively. The minimum association per cell was observed to be 2 IV + 7 II and was visualized in 3.33% cells. Shows the types of chromosomal associations in cowpea accessions and pooled chromosomal associations. Configurations of more than 4 chromosomes have not been observed, indicating that there is little if any role of gross structural changes in the evolution of different species. However, the occurrence of multivalent/univalent configurations in meiotic system is an indicator of hybridity involved in the origin of different cultigroups.

The Pollen mother cells observed for the study of meiosis in *Lablab purpureus* showed all the stages of meiosis *i.e* Leptotene, Zygotene, Pachytene, Diplotene, Diakinesis, Metaphase I, Anaphase I, Telophase I, Metaphase II, Anaphase II, Telophase II and tetrads were clearly observed. The Chromosome pairing behaviour and Chromosomal association at diakinesis stage in *Lablab purpureus* revealed maxmimum paring as bivalents. Since in *Lablab purpureus* 2n=22, therefore, 11 bivalents were observed in maximum number of cells i.e. 80% cells (Table 1). At Diakinesis stage the other chromosomal associations observed were 10 bivalents + 2 univalents, 9 bivalents + 4 univalents both in 8% cells. Also it was interesting to observe 12 bivalents in 4% cells i.e. showing 2n=24 (Fig 1) which has also been reported earlier by (Singh, *et al*., 2007). In case of *Lablab*, at Metaphase stage also some meiotic anomalies in maybe some virus infected plants (Atika Naz *et al*., 2008) were observed in the pollen mother cells *i.e.* single chromosome bridge and laggards during Anaphase stage.

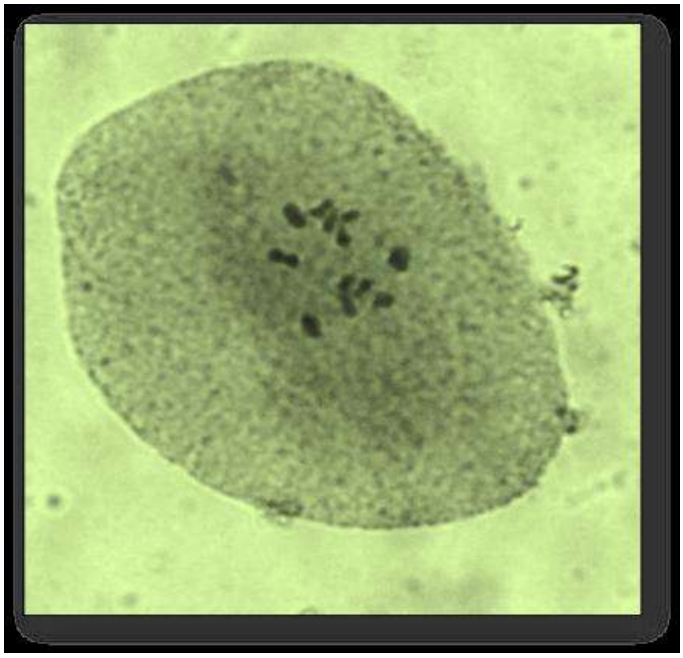

Fig 1: *Lablab purpureus* meiotic chromosome number variation 2n=24

Table 1. Types of chromosomal associations in Cowpea and Sem accessions

Cultigroups/accession	2n	Number of cells	Percent cells	Associations	Chromosomal associations range
sesquipedalis **EC 548875**	2n=22	1	6.66	2 IV + 7 II	IV II I 0-2 7-11 0-4
		2	13.33	9II + 4I	
		4	26.66	1IV + 8II+ 2I	
		6	40.00	1IV + 7II + 4I	
		2	13.33	11 II	
		Total = 15	Average association: 8.06 II + 0.8 IV + 2.67 I		
cylindrical **IC 438864**	2n=22	6	40.00	11 II	IV II I 0-1 8-11 0-4
		3	20.00	10 II + 2I	
		2	13.33	1IV + 8 II + 2I	
		4	26.66	9 II + 4I	
		Total = 15	Average association: 9.87 II + 0.13 IV + 1.73 I		
unguiculata	2n=22	1	6.66	1 IV + 8II + 2I	IV II I
		4	26.66	11 II	

IL-1177		6	40.00	10 II + 2I	
		4	26.66	9II + 4I	0-1 8-11 0-4
		Total =15	Average association: 9.87 II + 0.07 IV + 2.00 I		
unguiculata **EC 548999**	2n=22	5	33.33	1 IV + 8II + 2I	IV II I
		2	13.33	9II + 4I	
		3	20.00	11 II	
		1	6.66	10 II + 2I	0-2 7-11 0-4
		1	6.66	2 IV + 7 II	
		3	20.00	1IV + 7II + 4I	
		Total =15	Average association: 8.6 II + 0.7 IV + 2.13 I		

Pooled chromosomal Associations in Cowpea	**2n**	**Number of cells**	**Percent cells**
2 IV +7II	2n=22	2	3.33
9II + 4I		12	20.00
1 IV + 8II + 2I		12	20.00
1IV + 7II + 4I		9	15.00
11 II		15	25.00
10 II + 2I		10	16.66
		Total = 60	
Pooled chromosomal Associations in Sem	**2n**	**Number of cells**	**Percent cells**
10 II + 2I	2n=22	2	8.00
9 II + 4 I		2	8.00
11 II		20	80.00
12 II	2n=24	1	4.00
		Total = 25	

Conclusion

The chromosome number of 2n=2x=22 was found in all accessions, indicating lack of intraspecific variability for chromosome number in both the species. However, some meiotic anomalies and variability in chromosome number was also observed in negligible cases showing 12 bivalents in 4% cells i.e. showing 2n=24 maybe due to viral infection in Sem. Since relative basic information on cytological characterization of cowpea and Sem is not quite sufficient therefore, present investigation supplements to this information for two major fodder legumes, which can be used for conservation *i.e* maintaining purity as well as in genetic breeding programs of the species.

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