Table.3. A comparative account of the four varieties in terms of their characteristic features

Characteristic features	UPAS 120	ICPL 87119	NA 1	BDN 1
Maturity	It is an extra early maturing variety.	It takes 160 to 202 days for 75% maturity.	It takes late maturing variety.	It takes 155 -165 days for maturity.
Habit	Plants are medium tall, open and semi spreading type.	It is a medium -duration variety with an indeterminate growth habit.	It is a medium tall plant.	Plants are medium tall.
Stem	Long, erect, smooth and light green in color.	Green stem.	Light green stem.	Green stem.
Leaf	It has green trifoliate spirally arranged leaves along the whole stem. Leaves are quite large compare to other varieties.	Trifoliate green leaves.	Large expanded green leaves.	Large trifoliate green leaves.
Flower	It flowers vary profusely and normally the tips do not bear the pods. Standard petal of the flower is yellow with fine red streaks on it.	Flowers are yellow, with red streaks on the back of the standard petal. The pods are green with maroon streaks.	Yellow flower.	Flowers are yellow in colour.
Seed	Dark brown, Smooth, 7-8mm in size.	Brown, Smooth, 5 -7mm in size.	Light brown , Smooth, 4-5mm in size.	Light brown , Smooth, 5 -6mm in size.

Table. 4. Germination percentage of seeds after 7 days

Media	Name of the variety	*In vitro* percentage of germination (%)
MS basal medium	UPAS 120	50
	ICPL 87119	40
	NA 1	50
	BDN 1	30

Study of germination and growth of the different varieties under *in vivo* condition

After studying the percentage of germination of four varieties it was found that ICPL 87119 showed highest percentage of germination (90%) and BDN 1 showed lowest percentage of germination (55%) **(Fig.1)**.The growth pattern of different varieties was also studied and it was showed that ICPL 87119 had highest shoot length followed by UPAS 120, NA 1, BDN 1 and BDN 1 showed highest root length followed by UPAS 120, ICPL 87119, NA 1**(Fig.1)**.

Standardization of an *in-vitro* shoot regeneration technique for *Cajanas cajan*

Under *in vitro* condition, the percentage of germination was also studied, the germination percentage of BDN 1 was found to be lowest. UPAS 120, ICPL 87119, NA 1 exhibited 45-50% germination. UPAS 120 showed well response under *in vitro* condition. Except UPAS 120 no other variety showed further growth. For other varieties explants were taken from plants, germinated under *in vivo* condition. For UPAS 120, explants were selected from plants, germinated under *in vitro* condition **(Table.4)**. Petioles, leaves and hypocotyls were taken as explants for callus culture. Calli were formed in petioles after 15 days whereas they were observed in leaves after 10 days **(Fig.2)**.

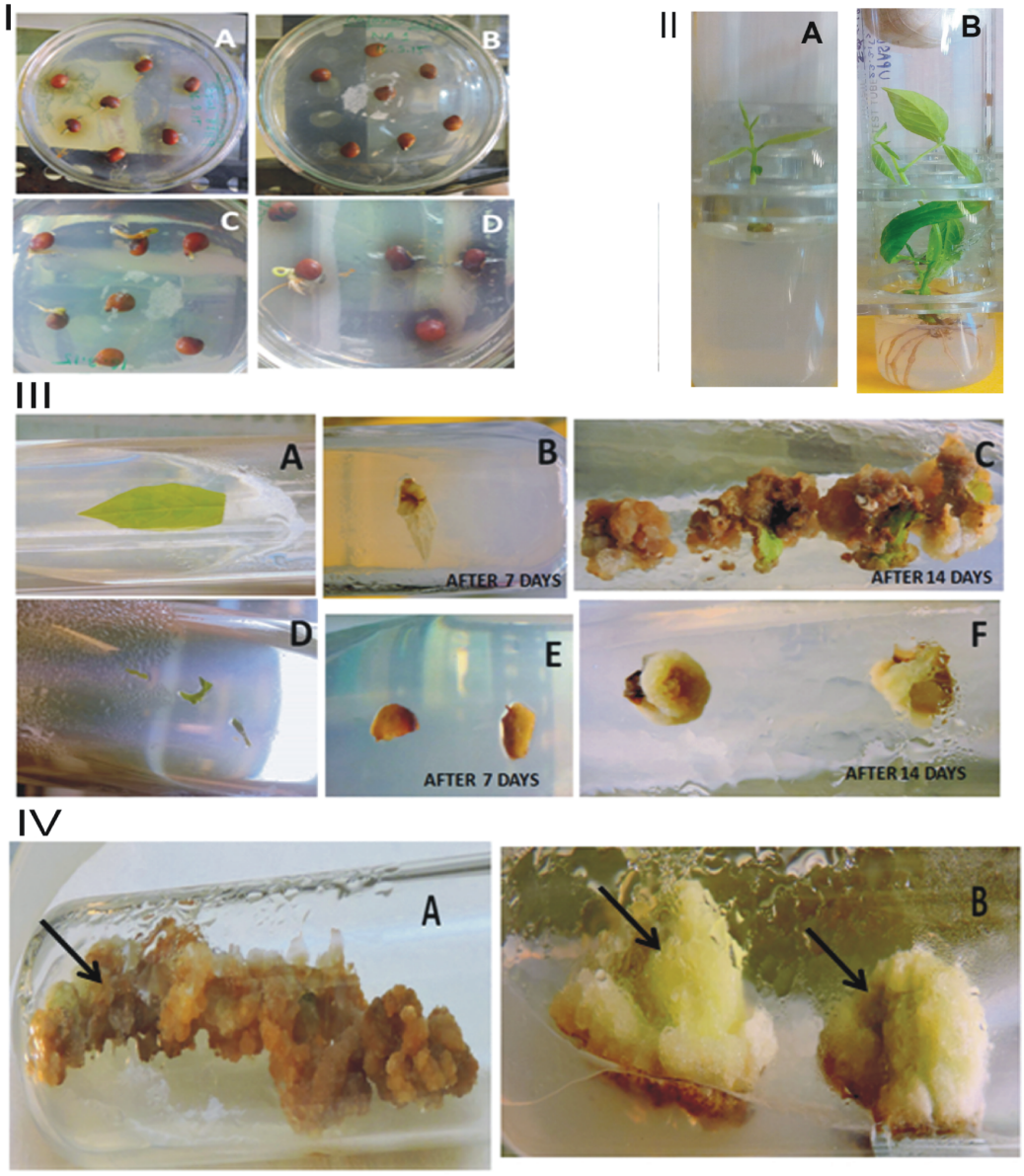

Fig.2.I *In vitro* **seed germination profile of the four varieties of** *Cajanus cajan*. **A. ICPL87119; B. NA1; C. BDN1; D. UPAS120. II.***In vitro* **grown plantlets of UPAS120. A. 1 week old; B. 2 weeks old. III. Standardization of callus formation and regeneration from different explants; A-C: leaf; D-F: petiole of UPAS120 variety.IV. Embryogenic calli showing regeneration in UPAS120 variety from A. leaf & B. petiole explants.**

The callus mass was compact, nodular, creamy white in colour. No calli were observed from hypocotyls. Callus was formed in the media containing BAP (4 mg/ml) and 2,4-D (0.5 mg/ml). We have standardized this media composition for callusing in UPAS 120. No callus was formed in other 3 varieties. So, calli of UPAS 120 were selected for further regeneration. Petiole and leaf calli were kept in regeneration media with different concentrations of PGRs. Organogenesis from induced calli was observed after 10days in MS media containing BAP (4mg/ml) and NAA (0.5mg/ml) **(Table.5)**.

Table.5. Standardization of regeneration media

Name of the explants	PGRs+MS media+3% sucrose				Observation
	BAP (mg/ml)	Kn(mg/ml)	IAA (mg/ml)	NAA (mg/ml)	
Petiole	2	2	-	-	No green callus was observed.
	4	-	-	0.5	Green callus were observed
	-	4	0.5	-	No green callus was observed.
	-	4	-	0.5	No green callus was observed.
Leaf	2	2	-	-	No green callus was observed.
	4	-	-	0.5	Green callus were observed
	-	4	0.5	-	No green callus was observed.
	-	4	-	0.5	No green callus was observed.

Study of chromosomal variation in callus tissue

Callus tissues are good source of chromosomal variation. Callus tissue may get such genomic heterogeneity possibly due to non-selective induction of asynchronous division of both diploid and endoreduplicated cells. Pre-existing genomic heterogeneity of explant may be a source of chromosomal variation in the callus tissue. The embryogenic calli showed several types of cells including non-dividing cells with small rounded nuclei, meristematic cells, which are small with densely stained cytoplasm and a prominent nucleus with condensed chromatin, and parenchyma cells, which are large and elongated. All these types of cells are characteristic of calli and have been previously described by Yeoman and Street (1973). Proper mitotic stages, from prophase to telophase were also observed in the 20 day old calli. The dividing cells those were fixed between 7am-10am are normal and more prominent. Metaphase chromosome (2n=22) were observed in the callus mass, fixed at around 9am. Callus from regeneration media showed globular cells with dense protoplasm. Metaphase stages, trichomes and glandular hairs were also observed **(Figure.3).**

Discussion

Cajanas cajan is a potential system which has economic importance, both in agriculture and medicine. It is also a good source to study intravarietal differences which may add an extra step in search of a good trait. The characteristic features of all the four varieties (UPAS120, ICPL 87119, NA1, BDN1) were compared in detail. A comparative study of the above said varieties of *Cajanas cajan* were done under *in vivo* and *in vitro* conditions.

In vitro techniques offer a sustainable and viable tool for rapid propagation, uniform progenies and disease free plants of desired genotypes. In the present study an attempt has been made to establish a proper *in vitro* micropropagation technique in the four selected varieties of *Cajanus cajan*. MS media (basal media) and modified MS media (supplemented with different PGRs) were used and standardized for callusing and regeneration. Callusing followed by callus mediated organogenesis was only observed in variety UPAS 120. Organogenic highly proliferative callus mass were obtained using the modified MS media using calli from petiole and leaf explants. We can also derive to conclusion that very high concentration of growth hormone proved to be toxic for plant. Being a good source, 20days old calli and regenerating calli were studied to see chromosomal variations. In the present paper we have standardized a protocol for germination and regeneration. This work is cost effective and need almost a year.

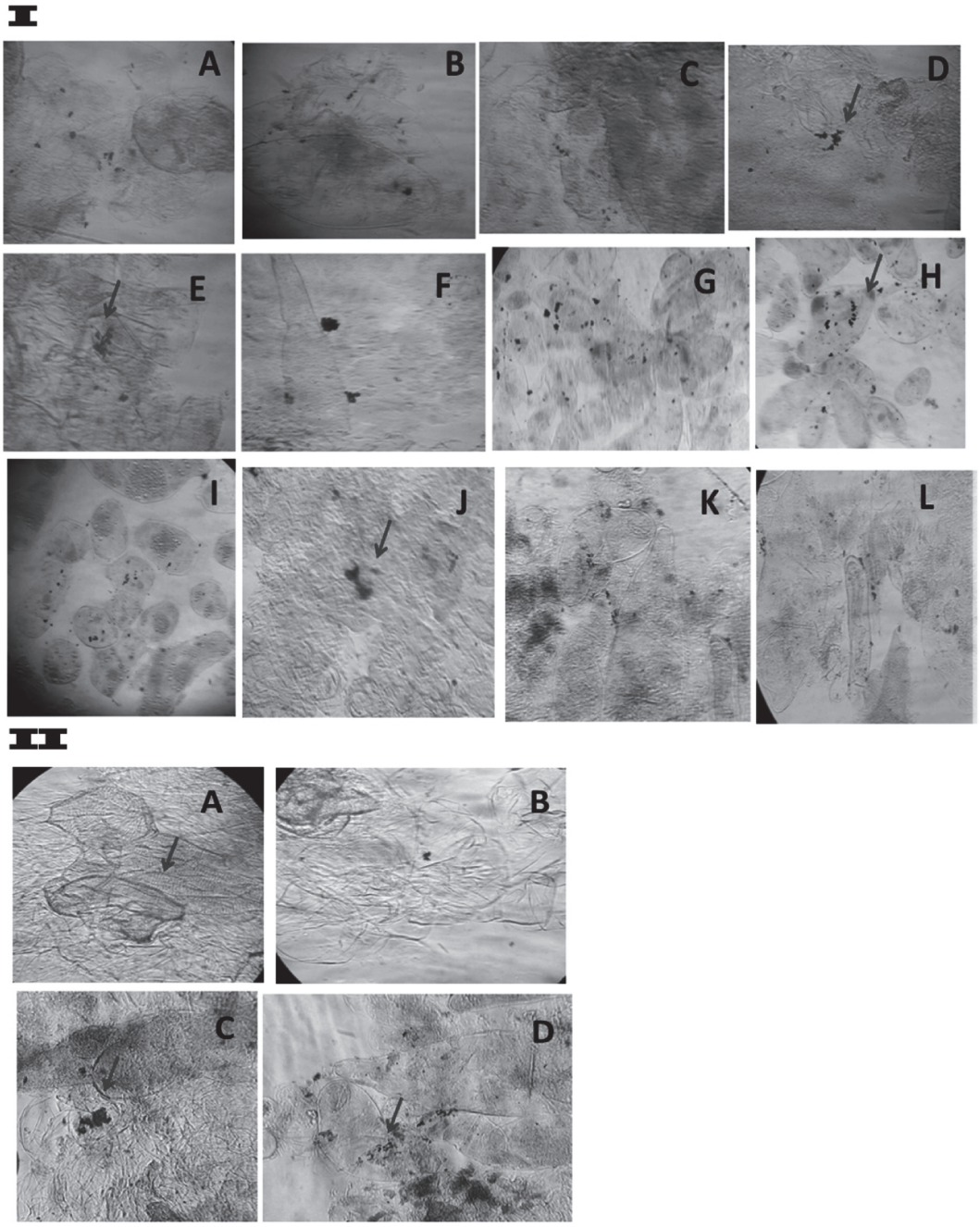

Fig.3. Study of chromosomal variation in callus tissue.I. Cytology of 20 days old calli (40X). The cells were fixed at A-B: 7am; C: 8am; D-E: 9am; F: 10am;G-L: 12 noon. Cells were irregular in shape, mostly elongated with mitotic aberrations like I: binuclei & vacuolation; J: double bridge at anaphase. Cells exhibit prophase (A-B;F) & metaphase (C-E) stages.

II. Cytology of one month old regenerating calli. The cells were fixed at 12 noon. A-B: shows trichome hairs; C-D: shows metaphase.

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