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Regeneration of Haploid Callus From Anthers of Pigeonpea

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Applications of haploid seedling are multiple; it is a first quality tool for studies in mutagenesis - also it is required for using hybrid vigour as a method of crop improvement. Of the many known methods of raising haploids (See Clapham, 1977), anther culture technique is most effective, simple and quickest route to haploidy. Since the first report of successful production of a haploid plant by anther culture in *Datura innoxia* (Guha and Maheshwari, 1964) this technique has been extended to nearly two dozen crop species (Nitsch, 1977). In pigeonpea, however, this technique has not yet been successful probably because, the methodologies for callus initiation and haploid plantlet regeneration are still not well established for this important pulse crop. The present communication reports our preliminary efforts of identifying optimal culture conditions for anther culture in pigeonpea, obtaining fast growing callus and regenerating haploid plants from the derived callus.

Unopened flower buds with anthers containing uninucleate microspores were collected from young fast growing plants of pigeonpea cultivar NP (WR)-15. Buds were surface sterilized for a minute with 0.1% mercuric chloride solution and rinsed with sterilized distilled water to remove traces of chlorine. Sterilized anthers were innoculated on to three different basal media viz., Modified White's medium (1954), Murashige and Skoog's medium (1962) and Nitsch's medium (1969) solidified with agar (0.8%) and supplemented with different concentrations of IAA, NAA and Kinetin. All the cultures were grown under cool white flourescent light (14 hr. day) at 24 ± 3° C.

Callus initiation was obtained after 50 days of culture on MS medium alone; supplemented with IAA (1.5 mg/l) and Kinetin (0.5 mg/l, Fig.1). White's and Nitsch's medium did not promote callus induction even after prolonged innoculation. Therefore, the treatments based on these two media were finally discarded.

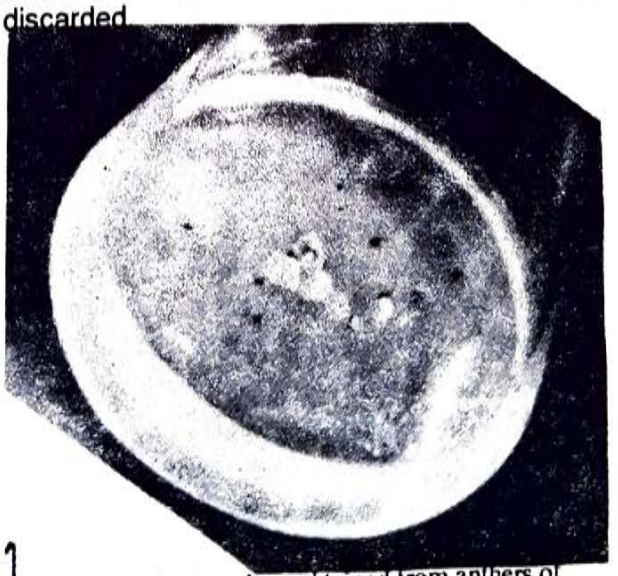

Figure 1. : Primary culture obtained from anthers of Pigeonpea on MS + 1.5 mg/l IAA + 0.5 mg/l Kinetin

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The primary culture obtained on MS medium was subcultured after 20 days on freshly prepared MS medium containing IAA (1.5 mg/l) and Kinetin (0.5 mg/l). Subculturing resulted in fast growing friable callus. Organogenesis was tried on these subcultured calli through manipulation of MS medium incorporating different concentrations of NAA, BA, IAA and Kinetin but the callus remained undifferentiated though showing prolific growth.

In order to confirm the ploidy level of this derived callus, chromosome counts were undertaken on primary and subcultured calli.

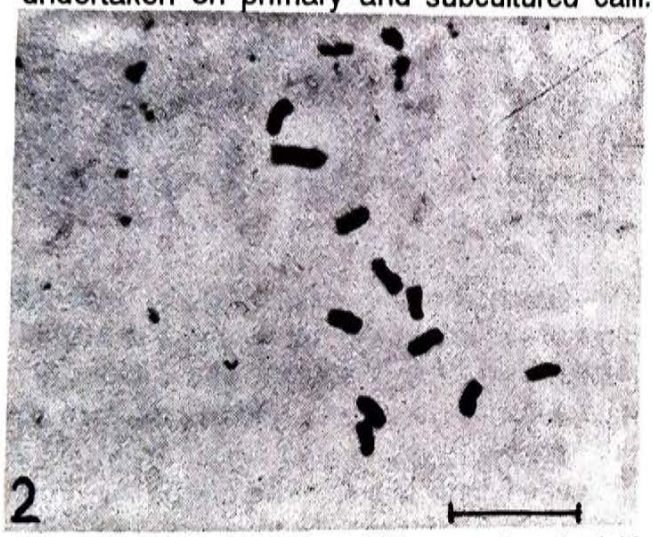

Figure 2. : 11 Chromosomes of Pigeonpea from haploid callus (x 2000) (Bar = 10 μm).

Method of Sharma and Sharma (1980) was adopted for chromosome studies. It was observed that in primary culture, callus retained its haploid nature (Fig.2) but in advanced subcultures the cytological changes occurred which lead to the development of polyploid and aneuploid cells and finally the tissue became mixoploid through endomitosis (Fig. 3).

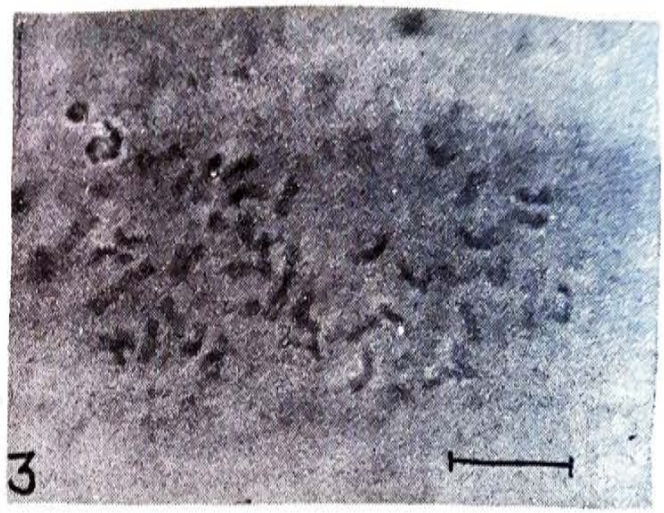

Figure 3. : A polyploid (4n) cell showing 44 chromosomes (x 1500) (Bar = 10 μm).

Looking to the foregoing observations, it seems that pigeonpea is quite conducive for regeneration, what only required is a further insight in to culture conditions. Efforts on these lines are underway in this laboratory.

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