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In vitro regeneration of pigeonpea from leaf with petiole explant

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ABSTRACT

The two genotypes TAT-10 and PKV TARA responded for direct regeneration, 90-100% establishment with good growth of explants of both the genotypes was recorded on all 21 media combinations under study. On shoot bud induction medium, MS + BAP 2.0 mg/l with 0.1 mg/l NAA , significantly higher average number of shoot buds / explant were recorded in both the genotypes. The shoot elongation treatment combination MS + 0.2 mg /l BAP recorded highest per cent conversion of shoot buds to shoot and significantly higher number of shoot elongation / clump of shoots. The rooting medium ½ MS + 1.5 mg/l IAA recorded significantly higher average number of secondary roots / shoot in the genotype TAT-10 and rooting medium ½ MS + 1.0 mg/l IAA recorded significantly higher average number of secondary roots / shoot in the genotype PKV TARA.The rooted plantlets of both the genotypes when hardened in a plastic cups on soilrite initially supplemented with ½ MS liquid medium and covered with polythene for a week showed 75% survival.

Key words : Pigeonpea, *In vitro* regeneration

INTRODUCTION

Pigeonpea is an important grain legume crop of rainfed agriculture in the semi-arid tropics. The conventional breeding methods are the most widely used for crop improvement. But in certain situations, these methods have to be supplemented with modern biotechnological techniques either to increase their efficiency or to be able to achieve the objective, which is not possible through the conventional methods. Transformation is one of the important technology developed which expands the sources of genes for plant improvement to all organisms, far beyond the gene pool accessible via sexual hybridization. Transformation also offers strategies for over expressing or suppressing endogenous genes. Thus, introducing new genes or manipulating endogenous gene expression via transformation generates new phenotypic variation useful for investigating gene function and for crop improvement.

Several attempts and number of reports yet, it has not been possible to achieve direct regeneration from leaf segments of *C. Cajan*, where most of the reports are on callus tissue (Eapen and Georg 1993, Georg and Eapen 1994).

MATERIALS AND METHODS

The experimental material of present investigation comprised of two varieties of pigeonpea *viz.,* TAT- 10 and PKV - TARA. The genetically pure seeds were obtained from Senior Research Scientist, Pulses Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. The leaf of 5-6 days grown seedling with petiole was used as explants. For preparation of explants mature seeds were used. The seeds were washed with 1% (v/v) lanoline followed by treatment with 0.1 % (w/v) mercuric chloride for 5 minutes,followed by rinsing with sterile distilled water for 5-6 times. The pre-sterilized seed were grown *in vitro* on ½ MS medium with 3% sucrose and 0.8% agar-agar. The 24-36 hrs. germinated seeds were cut to remove seed coat and cotyledons were splits open in a LAF cabinet. The embryo axes were extracted and the shoot apex region and the root pole were removed. The mature embryo axes in which both shoot and root pole were removed (referred to as decapitated mature embryo axes: DCMEA) were used as explants.

RESULTS AND DISCUSSION

At various stages of regeneration different media combination formulated using various concentrations of growth hormones (BAP, NAA, IAA and GA3) with Murashige and Skoog's (MS) basal medium were tried.

Shoot proliferation:

The leaf with petiole explant get increased in size after 7 days of inoculation. Genotype TAT-10 and PKV TARA showed 90.67 to 100 per cent establishment of leaf with petiole explant. The leaf segment and petiolar region enlarged and produced shoot buds at basal region of explants. The observations for initiation of shoot bud induction were recorded daily and data for number of multiple shoots induced was recorded on 30th day, after inoculation of explants. The explant failed to induce shoots on plain MS medium and in both the genotypes hence, all the treatment combinations tried were significantly superior over the control (Plate 1).

Out of different treatments tried for induction of

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