The Andhra Agric. J 60 (2):483-485, 2013

In Vitro Shoot Regeneration from Cotyledon of Redgram

Key words : Cotyledon, Organogenesis, Pigeonpea, Plant regeneration.

Pigeonpea (*Cajanus cajan* L. Millsp) is one of the most popular legume grains in the world, especially in the Indian subcontinent. Due to its multiple uses, pigeonpea is widely used in intercropping systems in semi-arid regions and ranks fifth in area after soybean, common bean, peanut and chickpea, and it is used in diverse ways as a source of food, feed, and fertilizer. It provides the main source of protein for many of the poorest populations and plays an important role in reducing malnutrition for millions of people around the world. It is consumed as a major source of protein to the human population in many developing countries. In India, it is the second important food legume contributing to 80% of the global production and it has been found in more than 30 countries in Asia, 37 countries in Africa, and across most of the countries of Central and South America and the Caribbean (Vander Maesen 2002).

Several biotic (Fusarium wilt, sterility mosaic and pod borer insects) and abiotic (drought, salinity and water logging) stresses, are serious challenges for sustainable pigeonpea production to meet the demands of the resource poor people of several semi-arid tropic regions of India and other countries. In recent years, genetically engineered resistance has been actively investigated in several crops. Genes conferring resistance to pests and diseases have been introduced successfully in a wide array of crop plants. It is imperative to have an efficient regeneration and transformation system in order to introduce novel traits in pigeonpea. Hence present investigation was planned to study the high efficient protocol for rapid *in vitro* plant regeneration from cotyledon of redgram.

Seeds of pigeonpea (*Cajanus cajan* L. Millsp) variety LRG-41 used in the experiment were obtained from plant breeding (pulses) section, Regional Agricultural Research Station, Tirupati and the experiment was conducted at Institute of Frontier Technology in the year 2011. In the experiment on better explants response and shoot regeneration there were 20 treatments and for each treatment four replications were made in a completely randomized design. Seeds of LRG-41 were Surface sterilized with 0.1% $HgCl_2$ and soaked in sterile water for overnight. The imbibed seeds were decoated and two cotyledons were carefully separated. Excised cotyledon was transferred to MS medium (Murashige and Skoog, 1962) supplemented at different napthalene acetic acid (NAA) 0-2 mg $l^{-1}$and 6-benzylaminopurine (BAP) 0-2 mg l^{-1}concentration. Explants were subsequently transferred in to regeneration media containing BAP and NAA maintained in light for 20days. There were seven explants per treatment and the experiments were repeated thrice.

Observations on reproducing explants and number of shoots were recorded 20 days after transferring to regeneration media. The regenerated shoots were transferred to the media with 0.4 mg $l^{-1}GA_3$ for shoot elongation. The cultures were incubated at 25±2°C under cool white fluorescent light (30µE. $m^{-2}.S^{-1}$) under continuous light for 3 weeks. The regenerated shoots were rooted on MS medium with different indole buteric acid (IBA) 0-2 mg l^{-1}concentrations. After getting maximum root length the plantlets were kept for hardening room at25±2^{0}C under diffuse light (16/8h photoperiod) conditions.

Type and concentration of plant growth regulators strongly influenced the organogenic potential of the cotyledonary explants of variety LRG-41. MS medium supplemented with various concentrations of BAP, which is most widely used and effective cytokinin for various legumes in combination with NAA. The cotyledons of the genotype LRG-41 cultured on MS media with cytokinin and auxin swelled and turned green after 20 days in culture producing small green dome like structures over the surface of the cotyledonary segment. After 3-4weeks of culture these structures developed in to shoot buds without intervening callus phase .The data on effect of concentration of MS media on in vitro shoot regeneration of redgram was presented in Table 1.

The average number of shoots per explants ranged from 2-3. Even though the initial response was high on most MS media with different concentrations of BAP and NAA. Among the various concentrations tested, 2.0 mg l^{-1}BAP and 0.1 mg l^{-1}NAA were found to be the best for maximum shoot

bud differentiation (Table 1). Percentage, as well as the number of shoots per explant showing differentiation of shoot buds was higher on MS media supplement with BAP and optimal BAP concentration for shoot regeneration was 2 mg l^{-1}.

Elongation of multiple shoots was obtained in MS medium with the concentration 0.4 mg l^{-1}gibberillic acid (GA3). Mehta and Mohan Ram (1980) observed that addition of GA_3 in the medium aided shoot elongation. Shiva Prakash *et al*., (1994) reported the elongation of shoot buds produced on the cotyledonary node which has pre-existing meristems and partially differentiated cells. Green healthy shoots regenerated were transferred to rooting medium. Though different concentrations of NAA and IBA were supplemented to MS medium, among them, Indole buteric acid (IBA) at 1.0 mg l^{-1}induced maximum frequency of rooting followed by NAA. Regenerated plants were successfully established in soil where 90 to 95% of them have been developed into morphologically normal and fertile plants. The regeneration of pigeonpea from cotyledons has been achieved previously (Mehta and Mohan Ram 1980; Kumar *et al*., 1983; Kumar *et al.,* 1984; Shiva Prakash *et al*., (1994); Naidu *et al*., 1995). George and Eapen (1994) also reported that formation of shoot buds on the distal ends of cotyledons when whole cotyledons were cultured. Patel *et al*. (1994) reported induction of somatic embryogenesis in cotyledons of pigeonpea when cultured on MS and B5 media supplemented with BAP, Kinetin and AdS.

Table 1. Effect of concentration of MS media on *in vitro* shoot regeneration of Redgram.

			No of explants Responding	Frequency of shoots	Mean no of shoot buds per explant	Mean no. of days taken for shoot initiation
BAP (mg l^{-1})	NAA (mg l^{-1})	GA_3 (mg l^{-1})				
0.0	0.0	0.4	NR	-	-	-
0.1	0.0		NR	-	-	-
0.5	0.0		NR	-	-	-
1.0	0.0		1	14.28	-	-
2.0	0.0		2	28.57	2	20
0.0	0.1	0.4	NR	-	-	-
0.1	0.1		NR	-	-	-
0.5	0.1		NR	-	-	-
1.0	0.1		2	28.57	4	21.5
2.0	**0.1**		**7**	100	**15**	20
0.0	0.5	0.4	NR	-	-	-
0.1	0.5		NR	-	-	-
0.5	0.5		NR	-	-	-
1.0	0.5		2	28.57	5	22
2.0	0.5		3	42.85	5	20.5
0.0	1.0	0.4	NR	-	-	-
0.1	1.0		NR	-	-	-
0.5	1.0		NR	-	-	-
1.0	1.0		2	28.57	3	19
2.0	1.0		4	57.14	6	21
0.0	2.0		NR	-	-	-
0.1	2.0		NR	-	-	-
0.5	2.0		NR	-	-	-
1.0	2.0		3	42.85	2	23
2.0	2.0		3	42.85	4	24
S.Em±				0.860	0.577	0.637
C.D@5%				0.179	1.729	1.829

NR: Not Responding * Number of Explants Per Petriplate is 7

It was concluded that the present work proved that efficient regeneration could be done with cotyledon as explant in redgram (var.LRG-41).The regeneration system described above would be useful for introducing new genes into the pigeonpea genome through *agrobacterium* mediated or particle bombardment transformation. De novo regeneration systems are amenable to *Agrobacterium*-mediated transformation. Since the present protocol fulfills the requirements for genetic transformation, transformation of explants to achieve transgenic pigeonpea is a possibility.

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(Received on 09.05.2012 and revised on 09.09.2012)