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Review Article

IN VITRO REGENERATION PROTOCOL FOR PIGEONPEA-A REVIEW

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ABSTRACT
Pigeonpea (*Cajanus cajan*) is mainly a tropical crop which is cultivated with the cereal grains such as maize, millet and sorghum etc. Pigeonpea as a valuable cover crop grown for food (dry or green seeds), feed (seed, leaves and young branches), firewood, medicine, fencing, roofing, shade and to make baskets. Inspite of large land covered for cultivation of pigeonpea there is a wide demand–supply gap as its production is constrained by various biotic and abiotic stresses. So attempts for development of an efficient *in vitro* regeneration protocol are made for conservation of this important legume. This review brings light to various culture conditions, explants and hormonal combinations to develop efficient *in vitro* regeneration protocol in pigeonpea.

Key Words: *Protocol, Legume Crop, Culture Conditions, Explants, In Vitro, Demand-Supply Gap*

Abbreviations
BAP, 6-Benzylaminopurine; GA_3, Gibberellic acid 3; IAA, Indole-3-acetic acid; IBA, 3-Indolebutyric acid; KIN, Kinetin; NAA, a-Naphthalene acetic acid; TDZ, Thidiazuron; MS, Murashige and Skoog; Ads, Adenine sulphate; ZEA, Zeatin.

INTRODUCTION
Pigeonpea (*Cajanus Cajan* [L.] Millispaugh*)* is an important grain legume of family Fabaceae. It is an out-crossed, diploid (2n=2x=22) crop with genome size of 800 Mbp. Pigeonpea is mainly cultivated in tropical and subtropical regions of the world. Globally pigeonpea is cultivated on 4.6 mh with annual production of 3.25 mt. India accounts for 78% of the global output with current production of 2.9 mt from 4.4 mh (Economic Survey of India, 2011-12). In India, pigeonpea is mainly grown in states of Madhya Pradesh, Uttar Pradesh, Rajasthan, Karnataka and Andhra Pradesh.

Pigeonpea is rich in protein (20-22%) particularly sulphur containing amino acids, namely methionine and cysteine (Singh *et al*., 1990). Besides, seeds also contain about 57.3-58.7% carbohydrates, 1.2-8.1% crude fibres and 0.6-3.8% lipids (Sinha, 1977). The pigeonpea varieties are broadly categorized into three classes based on duration of maturity *viz*. *early duration* (140-150 days), *medium duration* (160-200 days) and *late duration* varieties (more than 200 days).

It serves as a host for silkworm (Madagascar) and the lac insect. Stems and branches, especially those of medium- and long-duration cultivars, are used for basketry, thatching, fencing and as fuel. In Nigeria the stems serve as stakes for yam. Pigeonpea finds wide application in traditional medicine. Diarrhoea, gonorrhoea, measles, burns, eye infections, earache, sore throat, sore gums, toothache, anaemia, intestinal worms, dizziness and epilepsy are treated with leaf preparations, root preparations are taken to treat cough, stomach problems and syphilis, stem ash for wounds, and stalks and roots are chewed against toothache. Powdered seeds serve as a poultice on swellings. In Madagascar the leaves are used to clean teeth.

In post green revolution period, the per capita availability of pigeonpea has declined in the country mainly due to widening demand–supply gap caused by mismatch in population and production growths. In spite of being the largest producer of pigeonpea in the world, the average productivity in the country is mere 745 kg/ha. The major constraints that limit farmers from achieving potential yield of pigeonpea includes non-availability of quality seeds of improved varieties in adequate quantity, poor crop management, and biotic and abiotic stresses prevalent in the pigeonpea growing areas besides socio–economic factors.

The most important fungal diseases of pigeonpea are leaf spot (*Mycovellosiella cajani*,), *Fusarium* wilt (*Fusarium udum*), powdery mildew (*Leveillula taurica*), root-knot nematodes *(Meloidogyne* spp.) and reniform nematodes (*Rotylenchus* spp.). Insect pests like pod-sucking bugs (*Clavigralla* spp.), pod borers (*Helicoverpa armigera* and *Maruca vitrata*) and pod fly (*Melanagromyza chalcosoma*).

Among abiotic stresses, water logging during vegetative stage, cold sensitivity during flowering stage, terminal drought during grain filling stage and salinity/alkalinity throughout the crop period inflict major yield losses and instability in production. All these stresses make the crop less productive with unstable performance. Besides biotic and abiotic stresses, low initial crop growth and low harvest index also limit its yield production.

So, due to various stresses the production of this valuable crop is constrained therefore attempts for *in vitro* regeneration are done. *In vitro* regeneration protocol is affected by type of explants, genotype, hormonal combination and temperature and light conditions. This *in vitro* regeneration protocol also helps for development of transgenic protocol and production of transgenic plants.

Thus, the present review provides an overview of current knowledge concerning *in vitro* regeneration protocol in pigeonpea.

Pigeonpea Regeneration

Pigeonpea regeneration is affected by pathway of regeneration, genotypes, culture conditions and combination of growth regulator selected for developing the protocol. Effects of these parameters to develop a suitable protocol are given below:

Pathways of Regeneration

Various protocols for plant tissue culture have been developed during the past five decades and any plant species can now be regenerated *in vitro* through several pathways. Various pathways have been put forward to depict the regeneration processes such as organogenesis and somatic embryogenesis. Cells, tissues and organs from numerous plant species can be successfully cultured aseptically to regenerate whole plants.

Organogenesis

Organogenesis is the development of adventitious organs or primordia from undifferentiated cell mass or directly from the explant in tissue culture by the process of differentiation. Organogenesis usually involves induction of shoot buds leading to the development of shoots from the explant tissue, with or without an intervening callus stage, followed by transfer to a root induction medium for root formation and development.

The developmental stage and physiological state of the explant at the time of culture would affect the ability to induce direct organogenesis and differentiation without an intervening callus stage (Thomas and Davey, 1975).

For the induction of organogenesis *via* callus on a particular medium, the medium should cause dedifferentiation (callus induction), attainment of competence, induction for the organogenic pathway and determination for the pathway, and should not interfere with the morphogenic expression of the developmental pathway (Christianson and Warnick, 1985).

Therefore, organogenesis was found to be the most reliable pathway for the regeneration of transgenic plants.

Protocols for obtaining stable regenerants in pigeonpea have been reported through organogenesis from apical meristem (Cheema and Bawa, 1991), undifferentiated callus (Kumar *et al.*, 1983; George and Eapen, 1994), differentiated non meristematic tissues like leaf (Eapen and George, 1993; Eapen *et al*., 1998; Geetha *et al*., 1998; Singh *et al*., 2002; Dayal and Lavanya, 2003; Villiers *et al*., 2008) and various seedling explants such as hypocotyls (Geetha *et al*., 1998), cotyledons (George and Eapen, 1994; Geetha *et al*., 1998), cotyledonary nodes (Kumar *et al*., 1983; Geetha *et al*., 1998; Mehta and Mohan Ram, 1980; Shiva Prakash *et al*., 1994; Naidu *et al*., 1995; Kumar *et al*., 1984), epicotyls (Geetha *et al*., 1998; Naidu *et al*., 1995; Kumar *et al*., 1984) and embryonal axes (Franklin *et al*., 2000).

Organogenesis is of two types 1 Direct and 2 Indirect. Organogenesis by direct rather than indirect pathway is preferred due to the problems with somaclonal variations confronted in callus cultures (Ritchie and Hodges, 1993).

Direct Organogenesis

In direct organogenesis, either the shoot or root is induced directly from the pre-existing cells in the explant without undergoing an intervening callus phase (Brown and Thorpe, 1986, Christianson and Warnick, 1988).

Direct organogenesis *in vitro* was reported in pigeonpea (Geetha *et al*., 1998; Dayal and Lavanya, 2003; Shiva Prakash *et al.,* 1994; Mohan and Krishnamurthy, 1998; Geetha *et al.,* 1999).

Indirect Organogenesis

Indirect organogenesis involves an intervening phase of callus proliferation and growth, followed by shoots or root induction. Indirect organogenesis was reported in pigeonpea (George and Eapen, 1994; Eapen and George, 1993; Eapen *et al.,* 1998; Ramesh and Baldev, 1994; Thu *et al.,* 2003; Dolendro *et al.,* 2003).

Somatic Embryogenesis

The developmental pathway in which embryos have been induced to form from a somatic cell or group of somatic cells is referred to as somatic embryogenesis. In pigeonpea, somatic embryogenesis was first reported (Patel *et al.,* 1992) and subsequently its pathway of regeneration was demonstrated in three cultivars (Patel *et al.,* 1994).

The auxin (2, 4-D), which is provided in the initiation phase of embryogenic cultures, generally leads to the induction of cellular proliferation (callus induction) along the embryogenic pathway of development (Evans *et al.,* 1981; Normura and Komanine, 1985).

It was reported that somatic embryos are regenerated from diverse genotypes using various explants tissues such as mature seeds, shoot apices, intact seedlings, leaves, petioles, hypocotyls, epicotyls, cotyledonary nodes, cotyledons, internodes, roots, endosperm and cell suspensions (George and Eapen, 1994; Sarangi *et al.,* 1992, Nalini *et al.,* 1996; Sreenivasu *et al.,* 1998; Anbazhagan and Ganapathi, 1999; Mohan and Krishnamurthy, 2002; Singh *et al.,* 2003). Somatic embryogenesis is of two types –

Direct Somatic Embryogenesis

Somatic embryogenesis, which occurs directly from cells of the explant tissue without an intervening callus phase, is stated as direct somatic embryogenesis (Conger *et al.,* 1983; Raghavan, 1986).

Indirect Somatic Embryogenesis

Indirect embryogenesis is a common pathway in which somatic embryos get induced and develop from proliferated callus (Mc William *et al.,* 1974; Williams and Maheswaran, 1986). The explant most often used in indirect embryogenesis is the immature zygotic embryo (Merkle *et al.,* 1990; Finer, 1994).

Genotypes/cultivar

In order to regenerate a plant through *in vitro* culture, the choice of the genotype forms the most important factor (Ritchie and Hodges, 1993).

The response of particular cultivars within a species responds differently to *in vitro* culture (Brown and Thorpe, 1986), which further illustrate that the genetic component is highly influential on success of *in vitro* culture and plant regeneration (Koornneef *et al.,* 1986; Hodges *et al.,* 1986).

The major factor which influenced the organogenesis response in pigeonpea has always been the genotype. Differential frequency of regeneration ranging 43–77% from the leaf petiolar region of pigeonpea *cv*. ICEAP 00557, ICEAP 00020, ICPL 88039, ICPL 86012, ICEAP 00040, ICPL 87091, ICEAP 00554 and ICEAP 00053 (Villiers *et al.,* 2008) and 41–71% from *cv*. ICPL 91011, ICPL 88009, ICPL 84031, ICPL 87, ICPL 2376, ICPL 8705, ICPL 332, ICPL 85063, and ICPL 87119 (Dayal and Lavanya, 2003).

Interestingly using the same cultivar (ICPL 88039), under similar culture conditions a different regeneration efficiency of 83 and 46% was obtained (Dayal and Lavanya, 2003; Villiers *et al.,* 2008). By

using leaf explants of *cv*. ICPL 161, ICPL 88039 and UPAS 120 genotype-based regeneration was reported (Eapen *et al.*, 1998).

The regeneration frequency ranged between 21 and 43 shoots per explant in *cv*. BP 86-34, CC 2376, CC11295, Gaut-88-29, Gaut-89-8, and SPMA-4 (Shiva Prakash *et al.*, 1994) and 93.2 shoot buds in Hyderabad C (Geetha *et al.*, 1998) with cotyledonary node explants. Similarly, a genotype-dependent varying regeneration response of 4–13 shoots per explant from cotyledonary node tissue of ICP 8863, ICPH 8, Pusa 33 and UPAS 120 was observed. Genotype dependency for shoot regeneration has also been evidenced from embryonic axes of *cv*. VBN1, VBN2, SA1, and CO5 (Franklin *et al.*, 2000).

The regeneration response in similar culture media showed no variability from leaf explants between the genotypes, ICPL 93086 and Tanzania-7 (Tyagi *et al.*, 2001), hypocotyls explants of AL 15 and Hyderabad C (Cheema and Bawa, 1991; Geetha *et al.*, 1998) and distal cotyledonary segments of Gaut 82-90 and T-15-15 (Mohan and Krishnamurthy, 1998).

In contrast, the genotype-independent organogenesis-mediated regeneration was reported from cv. T-21, PT-22, T-Visakha-1, ICPL-87, N-290-21, T-15-15, and Gaut 82-90 with mature embryo axes and intact seeds as explant tissues (Naidu *et al.*, 1995). While the pigeonpea cv. BDN-1, BDN-2, ICP-7182, ICPL-87, ICPL-87119, and TV-1 promoted callus proliferation with distal cotyledonary segment explants (Mohan and Krishnamurthy, 1998).

Despite genotype-dependent organogenesis has been observed, the regeneration frequency *via* organogenesis can be improved either changing the auxin: cytokinin ratios and/or largely by addition of antioxidant phenolic inhibitors.

High somatic embryo regeneration (90–97.0%) was realized from pigeonpea cv. Gaut 82-90, Gaut 82-99 and T15-15 with cotyledon explants (Patel *et al.*, 1994; Mohan and Krishnamurthy, 2002). Using leaf tissue as an explant, a low regeneration frequency of 37 embryos from pigeonpea *cv*. Vamban-1 (Anbazhagan and Ganapathi, 1999) and 35, 33, 32, 31, and 31 embryos from Pusa 852, H 86-5, Pusa 609, Pusa 856, and Pusa 855, respectively (Sreenivasu *et al.*, 1998) were observed.

The effect of genotype on somatic embryogenesis was further evaluated by culturing the cotyledonary nodes of various pigeonpea *cv*. Pusa 853, ICPH 8, Pusa 33, ICP 151, RWL 19, UPAS 120 and ICP 8863 with a globular embryo formation of 21, 18, 18, 16, 9, 8, and 3 from each explant on the same medium, respectively (Singh *et al.*, 2003).

The germination frequency from globular embryos also varied in relation to pigeonpea cultivars such as NP (WR) 15 (70%) (Patel *et al.*, 1994) and ICPL 87 (3%) (Nalini *et al.*, 1996). These studies suggest that less responsive genotypes of pigeonpea for somatic embryogenesis can still be exploited for organogenesis mediated regeneration.

Culture Conditions

Regeneration of complete plant from a single cell in controlled environment or *in vitro* was initially attempted (Vasil and Hildebrandt, 1965). Components of a medium include inorganic macro and micronutrients, reduced nitrogen, carbon source, vitamins and growth regulators (Gamborg and Shyluk, 1981). Of these components, the concentration and ratios of the growth regulators have proven to be most essential for culture initiation and morphogenesis (Skoog and Miller, 1957; Vasil, 1988).

In addition to the nutritional value, reduced nitrogen (Halperin, 1966) and sugar component (Brown and Thorpe, 1986) further affect morphogenesis. Many researchers have put forward various compositions of a nutrient medium for the growth of plant tissue (White, 1942; Murashige and Skoog, 1962; Linsmaier and Skoog, 1965; Gamborg *et al.*, 1968).

Media composition using distal cotyledonary segment explants showed the highest regeneration with 57 shoot buds on EC6 medium (Maheswaran and Williams, 1984), followed by modified B5 (Mante and Boll, 1975), White (White, 1963), B5 (Gamborg *et al.*, 1968), LS (Linsmaier and Skoog, 1965) and MS media. Further, the B5 basal medium promoted higher regeneration from cotyledonary node explants than L2 (Phillips and Collins, 1979) and MS medium (Thu *et al.*, 2003).

Comparison of different basal media B5, MS, N6 (Chu, 1978), AAP (Pental *et al.*, 1982) and B (Beinsberger *et al.*, 1991) using cotyledon explants of pigeonpea and it was reported that B-medium gave optimal responses (Chandra *et al.*, 2003). The use of MS medium was most preferred medium for tissue culture based studies on wide plant species (Murashige and Skoog, 1962). The medium was formulated with the entire essential macro and micro mineral elements and hence has become the first complete medium designed for *in vitro* culturing of diverse plant species. To date, MS medium is the most widely used culture media in various tissue culture practices such as micropropagation, callus induction, regeneration etc. However, specific media for specific plant groups are also available; for example, BS and L2 media have been used for legumes (Thu *et al.*, 2003). In MS media inorganic salts are supplied in two groups as macro salts or nutrients and as micronutrients. The salts needed in higher amounts are called macronutrients, which include nitrogen, phosphorus, sulphur, magnesium, calcium and potassium. Nitrogen is mostly provided in two forms as nitrates and ammonium compounds. The salts needed in trace amounts are called as micro salts, which include boron, zinc, molybdenum, manganese, copper and iron etc. Carbohydrate is supplied usually as sucrose. Vitamins are required in trace amounts as they catalyze the enzyme system of the cells.

Role of Plant Growth Regulators

The essentiality of plant growth regulators concentration in culture media was determined (Skoog and Miller, 1957). The relative concentration is very critical for growth and morphogenesis. The ratio of cytokinin to auxin depicts the occurrence of changes in plants. The higher cytokinin to auxin ratio is found to be suitable for shoot regeneration. Usually, the following growth regulators were used in pigeonpea regeneration. Auxins like IAA (Dayal and Lavanya, 2003; Mohan and Krishnamurthy, 1998; Yadav and Padmaja, 2003), IBA (Geetha *et al.*, 1998; Shiva Prakash *et al.*, 1994), NAA (George and Eapen, 1994) and 2,4-D (Anbazhagan and Ganapathi, 1999) were used in various combinations with cytokinins like kinetin (Geetha *et al.*, 1998, Dayal and Lavanya, 2003; Villiers *et al.*, 2008; Mohan and Krishnamurthy, 1998), BAP (Geetha *et al.*, 1998; Shiva Prakash *et al.*, 1994; Mohan and Krishnamurthy, 1998; Pudukkotttai, 1998), TDZ (Eapen *et al.*, 1998) to promote cell division, regeneration of shoots and to enhance proliferation and growth of axillary buds. The gibberellins are commonly used for shoot elongation and somatic embryo germination. The ranges of hormones used were as follows: auxins (0.1-3.0 mg/L), cytokinins (0.1-3.0mg/L) and GA_3 (0.1-1.0 mg/L). Plant growth regulators although play a primary role in growth regulation rather than nutritional supplementation in plant regeneration and development (Slater *et al.*, 2003). Subsequently, several reports were available, a list of which is presented in Table 1.

Grafting of Regenerated Shoots

Regenerated shoots obtained after sub-culturing were grafted onto root stock either *in vitro* or glasshouse. This depends on the successful union of stock and scion, leading to continuity of vascular elements. Higher establishment of plantlets were reported as compared to direct rooted plants and produced more seeds. Successful grafting was reported by several workers with variable frequencies like thiram treated autoclaved sand (Dayal and Lavanya, 2003), red soil + vermiculate + farmyard manure (1:1:1) (Majumdar, 2004) and mixture of sand and vermiculite (1:1) in green house (Villiers *et al.*, 2008). But grafting was less practised in comparison to rooting and hardening. Moreover, the success rate of grafting was less satisfactory than rooting and hardening.

Establishment/Hardening of Plants

Seedlings that were grown in a greenhouse will need a period to adjust and acclimate to outdoor conditions, prior to planting in the garden. This transition period is called "*hardening off*". Hardening off gradually exposes the tender plants to wind, sun and rain and toughens them up by thickening the cuticle on the leaves so that the leaves loose less water. This helps prevent transplant shock; seedlings that languish, become stunted or die from sudden changes in temperature. Generally individual plants may be covered with clear plastic bags and irrigated daily with 2-3 drops of tap water or ¼ MS salts. After 7-10 days the bags may be removed, gradually over a period of 7-10 days.

Table 1: Pigeonpea regeneration reports

S. No.	Genotype	Explants	Hormone		Grafting	References
			Shooting	Rooting		
1.	AL 15 & Hyderabad C	Apical meristem, Hypocotyl	MS + B_5 + BAP/KIN	-	-	Cheema & Bawa,1991
2.	-	Mature cotyledons, Primary leaves and roots	-	½MS + B_5 + 0.2mg/L NAA	-	George & Eapen, 1994
3.	ICPL 161, ICPL 88039, & UPAS 120	Primary leaves	MS + B_5 + 1-2mg/L TDZ (or) + 0.1mg/L IAA MS + B_5 + 1mg/L BA + 0.1mg/L IAA + 1mg/L GA_3 (elongation)	-	-	Eapen *et al.*, 1998
4.	AL 15 & Hyderabad C	Epicotyls, hypocotyls, leaf, cotyledon and cotyledonary nodal segment	(a)MS + B_5 + 2mg/L BAP + KIN (differentiation) (b) MS + B_5 +1mg/L BAP + 0.1mg/L NAA (+) 1-5mg/L GA_3 (Proliferation and elongation)	MS + B_5 + 0.2mg/L IBA	-	Geetha *et al*., 1998
5.	ICPL (91011, 88009, 84031, 87, 2376, 8705, 332, 85063 & 87119)	Leaf	MS + B_5 + 5.0µM BA + 5.0µM KIN MS + B_5 + 5.0µM BA + 5.0µM KIN + 0.58µM GA_3 (elongation)	MS + B_5 + 11.42µM IAA	Small pots with autoclaved sand and thiram	Dayal & Lavanya, 2003
6.	ICEAP (00557, 00020, 00040, 00554 &00053) ICPL (88039, 86012 & 87091)	Leaf	MS + 5µM BA + 3% sucrose + 0.8% (w/v) agar MS + 5 µM BA + 3% sucrose + 0.8% (w/v) agar + 0.58 µM GA_3 (elongation)	MS + B_5 + 1% sucrose + 11.4 µM IAA	In pots containing mixture of sand and vermiculite (1:1) in green house	Villiers *et al*., 2008
7.	BP 86-34, CC 2376, CC11295, Gaut-88-	Cotyledonary node	MS+B_5+2mg/LBAP +MS+B_5+2mg/L	MS+B_5+0.5 mg/L IBA	-	Shiva Prakash *et al.*,

	29, Gaut-89-8 & SPMA-4		BAP+IAA			1994
8.	T-21, PT-22, T-Visakha-1, ICPL-87, N-290-21, T-15-15, & Gaut 82-90	Cotyledon, Epicotyl, Mature embryo axes, Intact seed	MS + B_5 + BAP + KIN	-	-	Naidu *et al*., 1995
9.	-	Apical meristem, Cotyledon, Epicotyl	MS + B_5 + BAP	-	-	Kumar *et al.*, 1984
10.	VBN1, VBN2, SA1 & CO5	Mature embryo axes	MS + B_5 + BAP + NAA	1/2 MS + 2.41 µM IBA	-	Franklin *et al*., 2000
11.	BDN-1, BDN-2, ICP-7182, ICPL-87, ICPL-87119, and TV-1, Gaut 82-90 & T-15-15	Cotyledon	(a)MS + B_5 + 22.2µM BAP + 2.3µM KIN + 271 adenine sulphate (b)MS + B_5 + 2.22µM BAP + 0.54µM NAA (or) ½MS + 2.89µM GA_3 (elongation)	MS + B_5 + 4.92µM IAA	-	Mohan & Krishnamurthy, 1998
12.	-	Germinated seedlings	B5 + 10 mgl^{-1} 6-BAP	B5 hormone-free medium	-	Thu *et al*., 2003
13.	-	Mature cotyledonary segments	MS + B_5 + AdS + BAP + KIN	-	-	Patel *et al*., 1994
14.	-	Leaf, Root, Epicotyle, Cotyledon	MS + B_5 + BAP/BAP + NAA	-	-	Nalini *et al*., 1996
15.	Pusa-606, 609, 852, 855, 856 & H-86-25	Leaf	MS + B_5 + TDZ	-	-	Sreenivasu *et al*., 1998
16.	-	Seedling leaf	MS + B_5 + 6.78µM (or) 4.52µM 2,4-D	-	-	Anbazhagan & Ganapathi, 1999
17.	T-15-15, GAUT-82-90, GAUT-82-99	Cotyledons	EC6 + BAP + TDZ MS + B_5 + 2.89-14.43µM GA_3 (Elongation)	½ MS + 0.38µM ABA	-	Mohan & Krishnamurthy, 2002
18.	-	Cotyledonary node	MS + B_5 + TDZ	-	-	Singh *et al*., 2003
19.	ICPL 93086, Tanzania-7 & F1	Leaf, Root, Shoot	KM medium + BAP + NAA + KIN	KM medium +IAA + 2IP +	-	Tyagi *et al*., 2001

	Hybrid			GA_3+ BAP		
20.	-	Cotyledon	MS + B_5 + BAP/TDZ/ZEA/ZEA riboside	-	-	Yadav & Padmaja, 2003
21.	ICPL 93115	Seedling leaf	(a)MS + B_5 + 5.0mg/L BA (b)MS + B_5 + 1mg/L BA (elongation)	MS + B_5 + 1.0mg/L IAA + 0.1mg/L KIN	-	Slater *et al*., 2003
22.	-	Apical meristem	MS + B_5 + 13.31 µM BAP	½MS + B_5 + 0.49-24.6 µM IBA	-	Pudukkotttai, 1988
23.	-	Shooting buds	MS + B_5 + 2-5mg/L BAP + 0.1-0.5mg/L NAA + 0.3mg/L IBA	MS + B_5 + 0.3mg/L IBA	Small pots with red soil + vermiculite + farmyard manure (1:1:1)	Majumdar, 2004
24.	-	Seed, Hypocotyl	-	-	-	Shama Rao & Narayanaswamy, 1975
25.	-	Anther	MS + B_5 + KIN + IAA	-	-	Bajaj *et al*., 1980
26.	-	Cotyledon, Mature embryo axes	MS + B_5 + BAP	-	-	Sarangi & Gleba, 1991
27.	-	decapitated embryonic axes	MS + BAP 1 mg L(-1) + IAA (0.5 mg L(-1)	MS + IBA (0.3 mg L(-1)	-	Rathore & Chand, 1999
28.	ICPL 88039	Cotyledonary node	MS + B_5 + 5 mg/l BAP + 3% sucrose + 0.8% agar [MS + B_5 + 2 mg/l BAP + 3% sucrose + 0.8% agar MS + B_5 + 1 mg/l BAP + 3% sucrose + 0.8% agar (Elongation)]	-	-	Ramchandar, 1999
29.	-	Cotyledonary leaves	MS + B_5 + 8.9µM BAP + 5.37µM NAA	-	-	Kumari *et al*., 2001

30.	Bahar & UPAS120	decapitated embryonic axes	MS + B_5 + IAA + BAP	MS + B_5 + IAA + BAP	-	Yadav & Chand .,2001
31.	ICP 26 & ICP 28	Leaf petiole	MS + B_5 + BAP + NAA	MS + B_5 + 0.3mg/l IBA	-	Srinivasan *et al*., 2004
32.	-	cotyledonary nodes	EC6 + 4.0 mg /L BA + 0.1 mg /L IBA	-	-	Qing-he *et al*., 2010
33.	LGG-29	Embryo	MS + 1.0mg/L BAP + 0.1mg/L NAA + 3% sucrose + 0.8% (w/v) agar MS + 0.4mg/L GA_3 + 3% sucrose + 0.8% (w/v) agar (elongation)	MS+1mg/L IBA	-	Guru Prasad *et al*., 2011
34.	ICPL 87-118 & ICPL151	mature cotyledon	MS + B_5 + 2.0 mg/l (2,4-D) or (TDZ)	MS + 0.1mg/l BA	-	Aboshama, 2011
35.	-	Leaf	MS + B_5 + NAA, IAA + IBA	MS + B_5 + NAA	-	Kashyap *et al*., 2011
36.	JKR105	Embryonic axes	MS + B_5 + 2.5 mg L^{-1} 6-BAP	MS + B_5 + 2 % sucrose + 0.5 mg L^{-1} 3-IBA	-	Krishna *et al*., 2011
37.	-	Callus	MS + B_5 + 1.0 mg l-1 IAA + 0.9 mg l-1 KIN	MS + 1.0 mg l-1 2,4-D	-	Prabhakaran *et al*., 2011
38.	AL 201	Cotyledonary nodal	MS + B_5 + basal MS, MS + 3 mg/l BAP + 1mg/l KIN and MS + 3mg/ l BAP + 1 mg/l KIN + 0.2% w/v charcoal media.	Basal MS medium , MS + 3 mg/l BAP + 1 mg/l KIN and MS + 3 mg/l BAP + 1 mg/l KIN + 0.2% (w/v) charcoal	-	Kaur *et al*., 2012
39.	LRG-41	Cotyledons	MS + B_5 + BAP + NAA	MS + B_5 + IBA	-	Raghavendra *et al*., 2012
40.	ICPL 87119 (Asha)	Mature zygotic embryos	MS + B_5 + 4.0 mg/L + 3.0 mg/L and 2.0mg/L TDZ.	MS+0.5-3.0 mg/L IBA and 0.5–3.0 mg/L IAA	-	Ugandhar *et al*., 2012

CONCLUSION

After, reviewing the available literature on pigeonpea regeneration thoroughly it was observed that cotyledonary node explant proved to be most suitable for *in vitro* regeneration as it showed high regeneration frequency. MS media was the most suitable medium for tissue culture based studies on several plant species. Among cytokinins, 2 mg/l BAP is the most preferred over kinetin (KIN) and thidiazuron (TDZ) due to high shoot bud regeneration. Usually, 0.3-1.0 mg/l IAA and IBA combinations were used for better rooting. The GA_3 was essentially used for shoot elongation and somatic embryo germination. It was also observed that hardening of plants was more preferred than grafting. It also reflected that genotype had wide impact on regeneration frequency so no particular genotype can be considered the most suitable. Therefore, to develop *in vitro* regeneration protocol organogenesis regeneration pathway should be selected using BAP for shoot bud regeneration, GA_3 for shoot elongation and IBA for rooting.

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