CHAPTER 2

REVIEW OF LITERATURE

Regeneration of plants in legumes is largely restricted to a few forage crops. Nevertheless, grain-legumes which constitute the chief food crops, for long remained retractable to available culture strategies due to their limited regeneration potential (Bajaj and Gosal, 1981; Gresshoff and Mohapatra, 1981; Mroginski and Kartha, 1984; Hammatt *et al.,* 1986; Huyghe, 1990).

Among the grain-legumes, in the genus *Vigna, V. aconitifolia* has been the main material for extensive tissue culture studies (Gill *et al.,* 1987a). In this species, detailed protocol have been worked out for regenerating plantlets from a number of somatic tissue explants (Bhargava and Chandra, 1983; Gill *et al.,* 1986; Godbole *et al.,* 1984), protoplast - derived cell colonies (Shekhawat and Galston, 1983) and cell-suspension culture (Kumar *et al.,* 1988). In addition, tissue culture studies have also been done in *Vigna mungo* (Gill *et al.,* 1987a) and *Vigna unguiculata* (*syn V. sinensis*) (Bharal and Rashid, 1980; Kartha *et al.,* 1981; Rains *et al.,* 1986; Gill *et al.,* 1987b; Pandey and Bansal, 1989). However, studies on *in-vitro* culture of *Vigna radiata* are summarized in Table-1 and the following conclusions can be drawn:

1. Almost every part of the plant produces callus cotyledons, cotyledonary node, hypocotyl, shoot apices and roots show good cell proliferation. However, regeneration of shoot buds varies with explant type. By using proper explants, it is possible to induce organogenesis or embryogenesis either directly from explants or from callus.

a) Direct shoot regeneration : Direct regeneration of shoots without an intervening callus phase has been achieved from shoot tips (Bajaj and Dhanju, 1979; Goel *et al.,* 1983a; Singh *et al.,* 1985; Mathews, 1987); cotyledons (Mathews, 1987; Patel *et al.,* 1991) and cotyledonary nodes (Mathews, 1987). The shoots were rooted and resulting plantlets were successfully established in soil.

b) Organogenesis from callus : Calli derived from hypocotyl and root undergo rhizogenesis only (Singh *et al.,* 1985). Shoot organogenesis has been reported from callus derived from primary immature leaves (Mendoza *et al.,* 1992) or from leaves of 7-d old seedlings (Patel *et al.,* 1991). Regeneration of shoots from callus derived from cotyledons, cotyledonary nodes, shoot tips and root segments has not been reported (Mathews, 1987; Patel *et al.,* 1991).

c) Pollen embryogenesis from pollen derived callus has been reported by Bajaj and Singh (1980). The anther cultured at uninucleate pollen stage have showed various types of development ranging from multinucleate, bicellular, multicellular and formation of embryoids. Out of 28.5% callused anthers, only 1.98% have shown pollen embryos. Regeneration of complete plants from anther derived callus has not been successful.

d) Protoplast have been isolated from root (Xu *et al.,* 1981), leaves (Goel *et al.,* 1983b; Joshi and Schieder, 1987) and hypocotyl callus (Gill *et al.,* 1987b) of *V. radiata*. Cell division in protoplasts leading to callus formation has also been achieved (Joshi and Schieder, 1987; Gill *et al.,* 1987b). Regeneration of complete plants from protoplast derived calli has not been demonstrated.

e) Somatic embryogenesis : Immature cotyledons and leaf explants have been reported to be the most responsive explants in culture producing somatic embryos in *V. radiata* (Eapen and George, 1990; Patel *et al.,* 1991). The somatic embryos have developed only upto globular (Patel *et al.,* 1991) or cotyledonary stages (Eapen and George, 1990). But, their germination into plantlets has not yet been achieved (Eapen and George, 1990).

f) Interspecific hybridisation between *V. radiata* x *V. mungo* , *V. radiata* x *V. glabrescence* and *V. radiata* x *V. angularis* have been tried and as these crosses are not compatible. Embryo rescue techniques have been used to raise hybrid plants (Ahn and Hartmann, 1978; Gosal and Bajaj, 1983 a,b; Chen *et al.,* 1990).

2. MS and B_5 media are frequently used, but MS medium has been found to be the best for growth responses of different explants and calli (Mathews 1987; Patel *et al.,* 1991). Reduction of MS inorganic salts to half has favoured induction of regenerable calli (Mendoza *et al.,* 1992). Liquid L-6 medium (Kumar *et al.,* 1988) has been used for induction of somatic embryos (Eapen and George, 1990). The basal media have also been supplemented with various additives, *viz.,* proline, ascorbic acid, citric acid, yeast extract, coconut water and casein hydrolysate. Addition of ascorbic acid and citric acid (100 mg/l each) have been used to prevent the browning of shoot tip callus during subcultures (Mathews, 1987). Proline (1-2 mg/l) and yeast extract (50 mg / l) in combination with NAA and BAP have induced regenerable calli (Mendoza *et al.,* 1992). Presence of casein hydrolysate (500 mg/l) and coconut water (70 ml/l) in the medium have increased the recovery of plants from the parental and hybrid embryos (Gosal and Bajaj, 1983b).

Various explants have shown differences in growth regulators requirement for caulogenesis and organogenesis. An auxin and a cytokinin are required for the induction of callus from various explants. Among cytokinins, BAP and KIN, and among auxins, 2,4-D, NAA and IBA have been used to initiate callus.

BAP in combination with auxin has supported callus growth better than KIN. BAP has also been found essential for the induction of somatic embryogenesis from leaf derived calli (Patel *et al.,* 1991). On the other hand, zeatin excels BAP in inducing shoot differentiation (Pal *et al.,* 1991). Auxins, such as 2, 4-D, picloram or NAA at high concentration in the initial

medium, have been used for the induction of somatic embryos from calli derived from immature cotyledons (Eapen and George, 1990). On the other hand, no or low concentration of auxin is required for better shoot organogenesis (Patel *et al.,* 1991).

3. *Vigna radiata* has shown geontype specific regeneration as indicated by studies on shoot organogenesis from leaf derived callus (Patel *et al.,* 1991; Mendoza *et al.,* 1992).

It is clear from the above account that regeneration from truely dividing callus cultures has been restricted to one or two genotypes and that too with low efficiency. Moreover, none of the earlier worker has studied the various factors, such as choice of genotype, the physiological state of the explant, media components and conditions during culture, which influence plant regeneration, in detail. Further studies are, therefore, required to define precisely these factors in order to improve regeneration efficiency in *Vigna radiata*, which, currently is recalcitrant.

Table 1. Studies on growth responses of explants of various genotypes of Vigna radiata

	Variety	Basal medium	Growth regulators (M)	Explant	Morphogenetic responses	Reference
1.	Vigna radiata x Vigna angularis	-	-	Hybrid embryo	Plant	Ahn and Hartmann (1978)
2.	ML-1,ML-5'G-65	MS	IAA ($1.1x10^{-5}$M) + KIN ($2.3x10^{-5}$M)	Apical meristem	Plantlets	Bajaj and Dhanju (1979)
3.	G-65,SML-32, Shining moong No. 1	MS	IAA ($1.1x10^{-5}$M) + 2,4-D ($9x10^{-6}$M) + KIN ($9.4x10^{-6}$M)	Anther	Callus and androgenesis	Bajaj and Singh (1980)
4.	-	B_5	-	Shoot tip	Plantlets	Goel et al., (1983a)
	-	B_5	-	Hypocotyl & Leaf	Rooting	
	-	B_5	NAA ($2.7x10^{-6}$M) + BAP ($4.5x10^{-6}$M) or IAA ($2.8x10^{-6}$M) + BAP ($6.6x10^{-6}$M) or IAA ($2.8x10^{-6}$M) + BAP ($8.8x10^{-6}$M)	Shoot tip Hypocotyl & Leaf	Callusing and Rooting	
5.	PS-7	-	-	Leaves	Protoplast	Goel et al., (1983b)
6.	Vigna mungo x Vigna radiata	MS	IAA ($5.7x10^{-6}$M) + KIN ($9.4x10^{-7}$M) + CW (70 mg/l) or CH (500 mg/l)	Hybrid embryo	Hybrid plantlets	Gosal and Bajaj (1983a,b)
7.	S-8 ML-5	MS PC-L2 Miller & Nitch	-	Cotyledon	Plantlets	Mathews and Rao (1984)
8.	LGG-127, TT-9E, ML-12, ML-26,	B5	2,4-D($4.5x10^{-7}$M) + KIN ($2.3x10^{-7}$M)	Hypocotyl and root	Callusing and rooting	Singh et al., (1985)
	K-851, PS-16, Li-8, PIMS-2, ML-5, T-44	B5	NAA ($1x10^{-6}$M) + BAP ($1.3x10^{-6}$M)	Sliced shoot tips	Shoot	
9.	ML-5	MS	BAP ($5x10^{-6}$M) + IBA ($2.0x10^{-5}$M)	cotyledon	callus and shoots	Mathews (1987)
		MS	BAP ($3.4x10^{-6}$M) + IBA ($6.1x10^{-6}$M)	Cotyledonary node	Shoots	
		MS	IBA + BAP (each $2.5x10^{-6}$ - $2x10^{-5}$M)	Root	Callus	
		MS & Miller	BAP ($5x10^{-6}$M to $2.5x10^{-5}$M) + IBA ($2.5x10^{-5}$M)	Primordial leaves	Callus	
		PC-L2, Miller, Blaydes, B_5	-	Shoot tip	Plantlet	
10.	Vigna radiata var. sublobata	MS liquid medium	BAP ($4.4x10^{-6}$M) + 2,4-D ($4.5x10^{-6}$M) + NAA ($5.7x10^{-6}$M) + 14% sucrose	Hypocotyl protoplast	Protoclones	Gill et al., (1987b)
		MS	2,4-D ($9x10^{-6}$M) + CW (15%) + sucrose 3%	Protoclones	Callus	
11.	-	Liquid B5	Dicamba (0.5-1.0 mg/l) + BAP ($4.4x10^{-7}$M - $8.8x10^{-7}$M)	Mesophyll protoplast	Multicellular colonies	Joshi and Schieder (1987)

		B5	Dicamba (0.5mg/l) + BAP ($4.4x10^{-7}$M)	Multicellular protoplast colonies	Callus	
12.	ML-5	MS salts + B_5 vitamins	NAA ($5.4x10^{-5}$M) or 2,4-D($2.25x10^{-5}$M) or Picloram (5 mg/l)	Immature cotyledons	Callus	Bapen and George (1990)
		I_6 Liquid medium	Picloram (0.1mg/l) + GA_3 ($2.8x10^{-8}$M) + CH (200 mg/l) + one of cytokinins (BAP, zeatin, 2-iP or KIN at $2.5x10^{-7}$M)	Immature cotyledon callus	Somatic embryos	
13.	Vigna radiata x Vigna glabrescens	MS	-	Immature hybrid embryo	Somatic embryos	Chen et al., (1990)
14.	MI-7-21 PIMS-4	MS	BAP ($6.7x10^{-6}$M) + 2,4-D ($2.2x10^{-6}$M - $4.5x10^{-6}$M) or NAA ($5.4x10^{-6}$M - $10.7x10^{-6}$M)	Leaf	Callus	Patel et al., (1991)
		MS	KIN ($9.2x10^{-6}$M and $13.5x10^{-6}$M) or 2-iP ($9.8x10^{-6}$M and $14.8x10^{-6}$M) + NAA ($5.4x10^{-7}$M and $1.3x10^{-6}$M)	Leaf callus	Shoot	
		MS	BAP ($2.2x10^{-6}$M - $4.4x10^{-6}$M) + 2,4-D ($2.2x10^{-6}$M)	Leaf callus	Somatic embryos	
15.	BI and T-44	LS medium	BAP ($8x10^{-6}$M - 10^{-5}M)	Cotyledons	Shoots and genetic transformation	Pal et al., (1991)
16.	M 79-9-82 Pag-asa-1 Pag-asa-3 MG-50-10A	Full or half strength MS salts + MS vitamins	BAP ($8.8x10^{-6}$M) + NAA ($5.7x10^{-6}$M) and 50 mg/l yeast extract + 4.0 mg/l L-Proline	Immature primary leaves	Callus	Mendoza et al., (1992)
	Pag-asa-1 and MG-10 A	1/2 strength MS inorganic salts + vitamins	BAP ($8.8x10^{-6}$M)	Immature primary leaf callus	Shoots	