CHAPTER I

INTRODUCTION

C O N T E N T S

(A) GENERAL IMPORTANCE OF THE CROP

Pigeonpea of Red gram (Cajanus cajan) (L.) Millsp), also known as 'arhar' in Hindi or 'tuver' in Gujarati is a fascinating crop with great potential. It is a short duration/perennial legume shrub, widely grown throughout the semi-arid tropic extending between 30'N and 30'S latitude on about 3 million ha; 85 per cent of which are in India with an annual production of 2.4 million tonnes (Ranga Rao and Ray 1985; Anonymous 1987).

The exact origin of pigeonpea has remained controversial. A tropical and subtropical legume, pigeonpea is said to be native of Africa, Asia and some south sea Islands (Whyte et al 1953). It has long gained recognition in the United Arab Republic and Madagascar (Stanton et al 1966) and said to be introduced from Africa not only to Brazil and West Indies (FAO 1959), but also to India (Tothill 1948). From India, it has been introduced to Australia, Ceylon, Gambia and Jamaica (FAO 1959). However, according to Linnaeus (1937), the East India is the primary centre of origin for pigeonpea. Vavilor (1939) has also reported India as the native. Krauss (1932) quoted the discovery of pigeonpea from Egyptian tombs of 2200-2400 B. C. Indeed further investigations have indicated that it may have

originated in the region between Egypt and Eastern Africa, and later to have become widespread particularly in India and other tropical and subtropical regions of the world (Purseglove 1968).

In India, Bihar, Maharashtra, Madhya Pradesh, Uttar Pradesh, Tamil Nadu, Karnataka, Rajasthan, West-Bengal and Gujarat are the major states growing this crop. Gujarat ranks fifth and fourth in total area (3,02,500 ha) and production (2,23,400 tonnes) respectively (Anonymous 1987). Bharuch, Baroda, Sabarkantha, Kheda, Surat, Valsad, Dangs, Panchmahal, Mehsana, Ahmedabad and Banaskantha are the major districts of Gujarat growing pigeonpea as a sole or inter crop under rainfed or irrigated conditions. In Bharuch, Baroda and Surat districts, this cash crop is replacing the cotton crop from the last few years.

Pigeonpea is rich in protein (ranges from 18.5 to 26.3% with 59.5 to 90.5 % true digestibility) and lysine (6.08 %) and provides carbohydrates including fibres, certain minerals and vitamins. It provides cheaper source of protein than the animal originated one for the poor classes of people and serves a balanced diet with cereals. Splitted as well as whole grains are boiled with water and liquid as well as semi-solid

preparations are consumed as food with cereals like wheat, rice, maize etc. whereas, green pods are used as vegetables in certain parts of the country especially in Gujarat. Dry leaves, pod-husks and waste of 'dal' preparation (Splitted pulse) are fed to milking cattle with other food stuff. The dry stalks are sued as fuel. It is one of the best deep rooted preferential rotational as well as a mixture crop with cereals. It ameliorates the soils in which it grows. It could fix upto 69 kg N/ha per season (J.V.D.K. Kumar Rao et al 1980).

Pigeonpea is different from most other pulses because of its parennial nature. Although normally grown as an annual shrub, the plants may be grown for several years and develop into small trees. Pigeonpea has great compensatory ability under adverse conditions. It gives additional yield after the first harvest if sufficient moisture is available and it has great flexibility in a wide range of cropping systems. Pigeonpea tolerates drought stress well and is thus an ideal crop for semi-arid tropics. It also grows well in the sub-humid tropics, and performs well in temperatures upto 38°C. However, it will not tolerate frost (Faris 1983).

The crop has a wide range of maturity periods. There are cultivars that mature in less than 100 days and others which take over 250 days to attain maturity. The time to maturity can be greatly affected by photoperiod and temperature. Thus there exist maturity types of pigeonpea for many different conditions (Narayanan et al 1980).

Despite its international importance, scientific studies of its production, improvement and utilization have been limited till 1973 when International Institute for Tropical Agriculture began at Ibadan in Nigeria. In India research work on pigeonpea improvement is now centred at the International Crops Research Institute for Semi Arid Tropics (ICRISAT) at Hyderabad.

The major objectives in legume improvement are to increase grain yield, contents of methionine and cystine, protein digestibility and to reduce anti-enzymes and seed-hardness. There has been no significant increase in pulse production during the last decade mainly because of their low static yield and susceptibility to various fungal and viral diseases (Bajaj and Gosal 1981). Yielding component of a crop reaches a plateau over a period of time. These plateaus in crop plants have been overcome by innovation of breeding

approaches (Simmonds 1979) namely hybridization and fertilizer inputs. Quantitative and qualitative improvement of the crop plants depends on the available gene pool and its manipulation. Finite gene pool can only yield a finite response (Simmonds 1983). Inherently low genetic variability of legumes caused by predominantly high degree of self-pollination has imposed limitations on using conventional plant breeding approaches (Bajaj and Gosal 1981). Hence, the use of unconventional methods in the induction of variability has assumed greater significance (Greshoff and Mahapatra 1981). Progress made in the last couple of decades recognized plant tissue and cell culture techniques as potentially valuable tools in crop improvement (Carlson 1975). The immense possibilities that these techniques offer have been emphasized by Scowcroft (1977), Murashige (1978), Bhaskaran (1985) and many others.

(B) PLANT TISSUE CULTURE : Techniques and Applications

plant tissue culture techniques have become a powerful tool for studying the basic and applied problems in plant biology. The potential impact of these novel and powerful biotechnology on genetic improvement of crop plants is significant. However,

a tissue culture system is also offers an ideal 'model' system, which allows one to investigate physiological, biochemical, genetic and structural problems related to plants. Moreover, the technique is being used also as an adjunct to more traditional means/methods for plant modification.

Plant tissue culture technique thus present new strategies for the development and improvement of agricultural crops as well as drug yielding and economically important plants through selective breeding, genetic modification, mutant selection and micropropagation of elite plants. Recent developments in recombinant DNA technology along with the isolation and culture of protoplasts have further made it possible to produce genetically engineered plants and have opened up unprecedented opportunities for the manipulations of genetic composition of the plants. Hopefully, in the years to come this may produce improved food and forest crop varieties and result in increased food and biomass production.

The regeneration of plants from cultured cells and tissues is a key step in the application of tissue culture methodology for plant propagation and improvement. The development of efficient protocols for reproducible,

high frequency plant regeneration from cultured tissues have, therefore, assumed great importance (Rao 1987). Plant regeneration via tissue cultures occurs through Organogenesis and Somatic embrogenesis.

b-1 Regeneration through organogenesis

Organogenesis can be obtained either by direct differentiation of shoot buds from explants or through callus formation in explants and subsequent formation of shoots and roots. Organ formation in vitro was reported as early as 1939 when White (1939b) observed shoot and root formation in callus cultures of carrot. Following these, many attempts were made with tissues of diverse plant species which led to the elegant work of Skoog and Miller (1957), who demonstrated that the balance between auxin and cytokinin determines the nature of organogenesis. Since then there have appeared numerous reports of chemical regulation of organogenesis from cultured tissues derived from different plant parts from a wide range of plant species as reviewed by Murashige (1978), Bhojwani and Razdan (1983), and others.

Formation of organs in culture is largely controlled by three main factors: the inoculum,

the medium and the environmental conditions (Thorpe 1980, 1982). The physiological age of the explant is a critical factor. For examples, Raju and Mann (1971) have demonstrated that in Echeveria young leaf explants initiated only roots, older leaves regenerated shoot buds and leaves of medium age produced shoots and roots.

The composition of the nutrient medium is an important parameter to be optimized to achieve plant regeneration. The major constituents of the medium comprise of inorganic and organic salts, carbon source, vitamins and plant growth regulators. In certain instances natural growth factors such as deprotenized coconut milk, fruit pulp and juice, malt extract and yeast extract, etc. are also incorporated. Auxins (IAA, NAA, 2,4-D) and 2-cytokinins (KN, BAP, Z, 2ip) are more often used in culture media and their concentration and ratio often determine the nature of growth and organogenesis/embryogenesis.

Several media have been developed by various investigators and are being used in studies of tissue culture (Mehta and Bhatt 1990), the striking difference in various formulations being essentially

in the quantity and form of nitrogen. A comparative account of the components of various nutrient media is given by Bhojwani and Razdan (1983) and Ozias-Akins and Vasil (1985). Normally, B_5 medium is used for legume cultures. In the present studies, however, instead of using B_5 medium entirely, salt composition of MS medium was employed along with vitamins of B_5 medium. The said modified MS (MMS) medium evoked good response from the Cajanus cajan cultures.

Likewise, seasonal variations (Fellenberg 1963), dissolved oxygen concentration in culture media (kessel and Carr 1972), quality and intensity of light (Murashige 1977), temperature (Hussey 1986, Fonnesbech and Fonnesbech 1980), and other factors also influence organogenesis in vitro. To cite just one example, the optimum light intensity for shoot differentiation in herbaceous plants is around 1000 lux, low being around 300 lux, and the high from 3000 - 10,000 lux (Murashige 1977). On the other hand, Ruta graveolens cultures produced roots of various length in darkness, while under continuous illumination they produced full green shoots (Abou Mandour 1982).

In organogenesis, by and large, root and shoot developments are often mutually exclusive, and a sequence of media changes is necessary to generate an entire plant.

Till recently legumes were considered recalcitrant. It is only in the last decade that significant advances have been made in regenerating plants from callus tissues of legumes (Hammatt et al 1987). Most progress has been made in forage crops in which regeneration from callus cultures occurs through somatic embryogenesis and or caulogenesis. Regeneration has also been demonstrated in leguminous trees. However, the large-seeded grain legumes (pulses) used mainly for human consumption have shown, in most cases, only very limited regeneration. Table-I.1 illustrates progress made in generation of some important legumes, through organogenesis as well as embryogenesis during the last decade.

Besides, rapid multiplication on a large scale of selected elite plants, tissue culture methods offer great scope to generate variability, very essential for inbred plant sps.like legumes. Several approaches are adopted as described below:

Table I-1 : Regeneration in important legume crops

Species name	Mode of regeneration				References
	Denova (direct)	Orgeno-genesis	Embryo-genesis	Plant-lets	
Arachis hypogaea		±			Mroginski and Fernandez 1980
	+	+			bajaj et al 1981a
		+			Mroginski et al 1981
	+				Atreya et al 1984
			+		Banerjee et al 1988, Hazra et al 1989
		-	+	+	Ozias Akins P 1989
		+		+	Mckantly et al 1990
			+	+	Sellars et al 1990
Cajanus cajan	+				Kusumkanta and Padmanabhan 1964
		+			Shama Rao and Narayananswamy 1975
			+ (Pollen)		Bajaj et al 1980
	+			+	Usha Mehta and Mohan Ram 1980
	+			+	Suresh Kumar et al 1983
		+	+	+	Anonymous 1987
			+ (Protoplast)		Kulkarni and Krishnamurthy 1989
	+			+	Srinath Rao and Basavakaj 1990
			+	+	Vaidyanath and Amrithsagar 1990
Cicer arietinum		+			Singh et al 1982b
		+			Rao and Chopra 1987, 1989
			+		Rao and Chopra 1989a
		+			Veena Sanghvan et al 1989
Crotolaria juncea			+	+	Rao et al 1982, 1985
			+	+	Prema 1988
Dolichos lablab	+			+	Sounder Raj et al 1991
Glycine max	+				Chang et al 1980
			+		Lipmann and lipmann 1984
			+	+	Lazzeri et al 1985, 1987, 1988
			+	+	Li et al 1985
			+	+	Ranch et al 1985
		+	+	+	Barwale et al 1986
		+			Wright et al 1986, 1987
			+		Ancelet et al 1988
			+	+	Finer 1988
			+		Hepher et al 1988
			+	+	Feng et al 1989
			+	+	Parrott et al 1989
			+		Christou and Yang 1989
	+			+	Seth mante et al 1989

(Contd.)

Species name	Mode of regeneration				Regerences
	Denova (direct)	Orgeno-genesis	Embryo-genesis	Plant-lets	
Glycine max			+		Tetu et al 1989
			+		Tran Dang Kien et al 1989
			+		Sellars et al 1990
Lathyrus sativus		+		+	Charyal and Maheshwari 1980, 1983
		+		+	Mukhopadhyay et al 1980
	+			+	Bhojwani and Mukhopadhyay 1986
	+			+	Ray et al 1991
Medicago sativa		+		+	Staverek et al 1980
			+		Santos et al 1983
			+		Chen et al 1987
			+		Meijer and Brown 1987
			+	+	Walton and Brown 1988
			+	+	Arcioni et al 1989
Phaseolus vulgaris		+			Zagorska et al 1982
			+		Martins and Sondahl 1984
		+			Westhuizen and Groenewald 1990
		+		+	McClean and Grafton 1989
		+		+	Franklin et al 1991
Pisum sativum		+		+	Mroginski and Kartha 1981
			+	+	Jacohson and Kysely 1984
			+	+	Kysely et al 1987
		+		+	Natali and Cavallini 1987
		+		+	Johanna and Eriksson 1988
		+		+	Lutora and Zabelina 1988
			+		Kysely and Jacobsen 1990
Trifolium pratense		+		+	Beach and Smith 1979
			+	+	Phillips and Collins 1980
Trigonella corniculata			+		Lu et al 1982a
T. foenum-graecum		+			Xu et al 1982
Vicia faba			+		Griga et al 1987
Vigna aconitifolia		+			Bhargava and Chandra 1983
			+	+	Shekhawat and Galston 1983, Krishnamurthy et al 1984
		+		+	Susan Eapen et al 1986
			+	+	Kumar et al 1988
V. radiata		+		+	Helena Mathews 1987
		+	+	+	Chen et al 1990
		+		+	Gulati and jaiwal 1990
		+		+	Patel et al 1991
V. umbellata		+		+	Raut et al 1989

b-2 Regeneration via Somatic Embryogenesis

Somatic embryogenesis is the most fascinating phenomenon which results in the formation of a complete plantlet from a single cell and is by far the most convincing demonstration of the totipotency of plant cells (Rao 1987). It is the process by which haploid or diploid somatic cells develop into differentiated plants through characteristic embryological stages without fusion of gemetes. This phenomenon was first observed in suspension cultures of carrot (Daucus carota) by Steward et al (1958) and in carrot callus grown on an agar medium by Reinert (1959). Embryo formation is subsequently to occur either indirectly from callus/ cell suspensions or directly from an organized structure, i.e. organ explants. The induction and development of advantitious embryoids in cultures have been described in a number of reviews; e.g. Street (1975), Vasil and Vasil (1980), Evans et al (1981), Ammirato (1983), Raghavan (1983), Lutz et al (1985), Williams and Maheswaran (1986), and Durzan (1988).

Somatic embryo has been defined as a nonzygotic embryo arising from a single cell with no vascular

connections with maternal tissues (Street and Withers 1974, Haccius 1978). However, Raghavan (1976) and Tisserat et al (1979) demonstrated that in many cases apparently normal bipolar embryoids are formed from aggregates of cells. Thus, an embryo is ab initio a bipolarized entity (Rangaswamy 1986) bounded by cuticle, since physical or physiological isolation of the cell/cell group is considered prerequisite for them to embark upon the embryogenic pathway (Handro et al 1973, Street 1979, Vasil and Vasil 1982, Kononowicz et al 1984).

Somatic emrbyogenesis provides many advantages, e.g. production in large numbers (60,000 per liter of medium), the presence of both root and shoot meristems in the same unit, easy scale-up, transfers and other manipulations. Besides, embryos are 'natural organs of perennation, many of which typically become dormant. If dormancy could be induced in somatic embryos, the possibility arises that they could be incorporated into artificial seeds either by coating or encapsulation (Durzan 1980). In order to release improved varieties for production rapidly and effectively, an artificial seed engineering technique has been established for

carrot (Zhan 1984). Further, tissue culture derived "artificial seeds" offer scope for michanisation and automation for planting (Durzan 1980, Sharp et al 1982). This technique is also suitable for seed storage and shipment.

Population of somatic embryos also provide an important resource for the analysis of molecular and biochemical events that occur during induction and maturation. Some work has been reported along these lines e.g. the formation of developmental (temperature sensitive) mutants in carrot cultures (Breton and Sung 1982; Terzi et al 1982), isolation of embryo specific proteins (Sung and Okimoto 1981, 1983) and even stage specific storage proteins (Crouch 1982).

b-3 Somaclonal variation

Plants regenerated from established culture (callus, suspension) are frequently found to be variable. The callus and suspension cultures exhibit high chromosomal instability resulting into polyploids and aneuploids. This is attributed to heterogeneous population of cells in differentiated tissues. Chromosomal mossaics in callus and cell suspension cultures have been well documented (Sunderland 1977; Ammirato 1978). Long term cultures

of callus often result in changes in the Karyotype of the plant and the regenerated plants show variation for various characters.

Similarly, long-term grown callus mediated somatic embryogenesis is ideal for recovering useful variants (Lutz et al. 1985, Durzan and Gupta 1987, Tulecke 1987).

Somaclonal variations are already having some impact on the improvement of sugarcane and potato cultivars and in the breeding of new floricultural varieties. Increasing role of somaclonal variation as an adjunct to conventional plant improvement is envisaged. Recent research has demonstrated the role of such requirement realised in previously recalcitrant crop plants such as wheat (Shimada and Yamada 1979), rice (Oono 1978b) and important legumes (Bingham et al 1975; Beach and Smith 1979; Phillips and Collins 1979; Kao and Michayluk 1980; dos Santos et al 1980; Saka et al 1980 and Bhojwani and Mukhopadhyay 1986). Still, the frequency of regeneration through organogenesis in legume callus is generally not high enough for the screening of novel variants. Recent successes with somatic embryoids via legume callus offers greater

opportunity for employing the *in vitro* selection techniques. As a result there are not many reports on somaclonal variants from legumes except in *Medicago sativa*, and soybean (Latunde-Dada and Lucas 1988, Freytag et al 1989).

b-4 *In vitro* cellular selection technique

The greatest application of somaclonal variation for plant improvement is in selection for desirable mutations at the cellular level. Cellular selection is conceivable for the recovery of variants resistant to antimetabolite such as amino acid analogues, antibiotic drugs, pathotoxins, herbicides and physiological stress (Maliga 1978; Thomas et al 1979, Brettell and Ingram 1979, Nabors et al 1980; Selevapandiyan et al 1988 and Unnikrishnan et al 1991). Similarly, cellular selection for many agronomically important attributes are known; resistance to host specific toxins such as those found in *Drechslera*, *Pseudomonas* and *Alternaria* pathogens, tolerance to salinity, metal toxicity, temperature stress and waterlogging.

In vitro cellular selection is thus a tissue culture technique used to recover desirable mutant plants, including disease resistant plants. The

selection agents usually employed in selection of disease resistant cells are phytotoxic extracts. These extracts are incorported into the suscpension or semi-solid media used to culture the callus and/or explants. Plants regenerated from such resistant cells have expressed disease resistance (Hartman 1984). This approach, although extremely time saving and accurate, normally requires the use of a host specific fungal toxin.

Table I-2 illustrates progress made in *in vitro* cellular selection techniques in a number of plant species to isolate clones resistant to several fungal toxins.

b-5 Pathological studies with pigeonpea

More than 50 disease have been reported to affect pigeonpea (Nene et al 1984). Among these, wilt caused by *Fusarium udum* Butler., *F. solani*, *F. oxysporum* and *F. acuminatum* and sterility mosaic are important disorders. Wilt of *F. udum* is one of the most important diseases. The said wilt occurs in India in the range of 0 to 95 per cent with an average of 22 per cent (Kanniyan et al 1980). In Gujarat, this disease has been noticed upto 0 to 96 per cent (Anonymous 1983). Generally, wilt occurs

Table-1-2: *In vitro* selection for disease resistance

Species name	Pathogen	Selective agents	Stage of selection	Stage of resistance	Reference
Apium graveolens	*Fusarium oxysporum*	Gene	Cell	Progeny	Health-pagliuso and Rappaport 1990
		Gene	Cell	Progeny	Pullman and Rappaport 1983
Brassica napus	*Phoma lingam*	CF	Callus	Regenerate plant	Sacristan 1982
	Plasmodiophora brassicae	Sporen	Embryo	Genetic nature	Sacristan and Hoffmanu 1979
Dianthus caryophyllus	*Fusarium oxysporum*	CF	Callus	Callus	Bulatti et al 1985
Glycine max	*Phialophora greata*	CF	Callus	Callus	Gray et al 1986
	Phialophora gregata	CF	Cotyledons	Callus	Willmot et al 1989
Hordeum Vulgare	*Helminthosporium sativum*	Toxin	Callus	Callus	Chawla and Wenzel 1987
		Toxin	Cell	Progeny	Wenzel and Foroughi-Wehv 1990
Lycopersicon esculentum	*Fusarium oxysporum*	CF	Cell	Genetic nature	Scala et al 1984
		Toxin	Callus, Protoplast	Genetic nature	Shahin and Spirey 1986
	Alternaria solani	CF	Callus	Regenerate plant	Shepherd 1986
	Phytophthora infestans	CF	Callus	Callus	Ilig and Dallacgua 1986
		CF	Callus	Callus	Warren and Routley 1970
Medicago sativa	*Fusarium oxysporum*	CF	Callus	Regenerate	Arcioni et al 1987
		CF, Toxin	Cell, Callus	Progeny	Ignatora et al 1988
		CF	Cell	Regenerate plant	Binarova et al 1990
		CF	Callus	Regenerate plant	Hartman et al 1984

(Contd.)

(Contd.)

Species name	Pathogen	Selective agents	Stage of selection	Stage of resistance	Reference
Medicago sativa	Verticillium albo-atrum	Toxin	Protoplast Callus	Regenerate plant	Latunde Dada and Lucas 1983, 1988
		CF	Cell	Embryogenesis	Yu et al 1990
Nicotiana tabacum	Fusarium oxysporum	CF	Cell	Regenerate plant	Selvapandiyan et al 1989
	Alternaria alternata	Toxin	Callus	Genetic nature	Thanutong et al 1983
	Pseudomonas tabaci	Toxin	Cell	Genetic nature	Carlson 1973
	Phytophthora parasitica	Sporen	Callus	-	Haberlach et al 1978
Oryza sativa	Helminthosporium oryzae	Toxin	Callus	Genetic nature	Ling et al 1985
Solanum tuberosum	H. infestans	CF	Callus	Regenerate plant	Behnke, 1980, 1979
	Fusarium oxysporum	CF	Callus	Regenerate plant	Behnke 1980b
	Alternaria solani	Gene	Protoplast	Regenerate plant	Matern et al 1978
Saccharum offcicinarum	Helminthosporium sacchari	Toxin	Callus	Regenerate plant	Heinz et al 1977, Larkin and Scowcroff 1983
Trifolium Pratense	Fusarium roseum	CF	Cell	Regenerate plant	Constabel 1989
Vigna radiata	Xanthomonas campestris	CF	Callus	Callus	Singh and Thind 1988
Zea mays	Helminthosporium maydis	Toxin	Callus	Progeny	Brettell and Thomas 1980, Brettell et al 1980b Gengenbach et al 1977

at seedling and flowering stage and exhibiting wilting and drying, resulting into heavy losses. It alone causes an annual loss of Rs. 37 crores in India (Kanniyan et al 1984). Fusarium spp. attack the root of the plant and turning it brown to black. Roots being unable to absorb water, plant shows as a result shortage of the moisture and wilting symptoms. Gradual and sometime sudden yellowing, withering and drying of leaves followed by the drying of the entire plant are the prominent symptoms. It may result from injury to the root system, partial plugging of water conducting vessels by fungal mycilium or toxic substances secreted by the pathogen. This soil borne disease, sometime survives in host tissue on bodies of termites (Upadhyay and Rai 1982) and in some cultivar internally as in the seeds (Anonymous 1984). Therefore, it is difficult to control the wilt of arhar. The chemical control, if possible, will not be economical nor advisable.

Fusarial wilt of pigeonpea was first reported from India by Butler in 1906 (Butler 1906). Further, he described this pathogen as a new species and named it as Fusarium udum (Butler 1910). Later on, its occurence was reported from other countries

(Booth 1971; Nene 1980). Attempts were made by several workers to rename as Fusarium oxysporum f. sp. Udum but views of Booth (1971) found on sound footing and he preferred F. udum Butlet. Kanniyan and Nene (1979) reported F. oxysporum, F. acuminatum and F. solani in association of F. udum on wilted tissues of pigeonpea.

b-6 Mechanisms of plant resistance to wilt

Resistance to wilt disease may be controlled by a single dominant gene, as in the case of melon, pea, tomato, cabbage, sunflower and upland cotton or by two or more dominant genes as in Egyptian cotton, bananas, sweet potatoes and elms (Bell and Mace 1981). Resistance mechanisms might work in the host plant any time between spore germination on the plant surface to colony formation in the xylem vessels (Harling and Taylor 1984). The resistance effective against one wilt fungus is also effective against others. Thus resistance of soybean to Cephalosporium is also effective against Verticillium (Tachibana 1971). Similarly, resistance of cotton to Verticillium is also effective against Fusarium (Bell and Mace, 1981).

The resistance of the plant varies depending on the age of the plant. The juvenile resistance of elms to Ceratocystis is lost largely during the second growing season and mature trees reach a peak of susceptibility (Ceroselli and Feldman 1951, Schreiber 1970, Tounsend 1971). In pigeonpea, wilt generaly inflicts at different growth stages (Kannaiyam and Nene, 1981).

b-7 Sources of Resistance of Wilt disease

Despite the difficulty in isolating host genes that would confirm absolute resistance to the pathogen, breeders have produced several tolerant varieties to Fusarium spp. by conventional breeding techniques. In India, attempts were made to identity wilt resistant lines in 1905 at Poona (Butler 1908) and thereafter, several reports are available on screening for pigeonpea wilt. Baldev and Amin (1974) found C-11, NP (WR) 15 and S-103 as resistant. Singh and Misra (1976) found Bori-192-12-5-1-2 and Boari-192-15-2-11-42 as moderately resistant and Bori-193-5-5-12-42-13 and KWR-1 as tolerant. Earlier reported resistant varieties, viz. C-11, C-28, C-36, F-18, NP (WR)-15, NP-41 and T-17, were found to be susceptible in their intensive screening

programme. BDN2 and ICP.8862 are reported moderately resistant and moderately susceptible to wilt, respectively in field conditions (Anomymous 1978-79). Nene and his associates (1980) from ICRISAT reported ICP-1681, -3753, -3782, -4964, -5097, -6837, -7118, -7120, -7182, 7198,-7201, -7273, -7336, -7867, -8585, -8859, -8860, -8861, -8862, -8863, -8864, --8865, -8867, -8868 and ICP-8869 as the resistant entries against wilt. T-15-15 and ICP-8860 were observed as immune at S. K. Nagar (Gujarat Agricultural University) but both were observed susceptible at other locations of Gujarat (Patel et al 1981). Nene and Kanniyan (1982) screened out thousands of entries and recorded only 33 entries as resistant in field conditions and noted only one entry ICP-8863 as resistant in both glass house as well as laboratory tests. Jain et al (1983) screened 92 pigeonpea lines and observed 19 lines as resistant while rest showed the susceptible reaction with 3.0 to 59.0 per cent wilt incidence. Varieties, viz. ICPL-25, ICPL-31 and ICPL-108 were found resistant in wilt sick plot at ICRISAT (Kanniyan et al 1983). BDN-22, ICP-8863, T-15-15 and T-21 showed 20.9, 55.35, 87.23 and 96.83 per

cent wilt incidence respectively in wilt -sick plot (Anonymous 1983-84). ICP-616, -1680, -4784, -6654, -6974, -11308, -11324, -11368 and ICP-11405 showed resistant reaction to wilt, caused by F. udum for four consecutive years in vertisol wilt-sick plot at ICRISAT (Anonymous, 1985). Konda et al (1986) recorded ICP-8863 as highly wilt resistant variety against F. udum. Zete et al (1986) from Gujarat observed the cultivar BDN-2 as the tolerant to wilt. Zote et al (1987) in their studies observed BDN-1, 15-3-3, ICP-7336, ICP-8862 and AWR-74115 as resistant and BDN-2. ICP-8861, ICP-8860, ICP-8864 and Bandapalera as moderately resistant varieties to F. udum. Gupta and Gupta (1988) in their vigours testing recorded ICP-8863, ICP-10957, ICP-10988, ICP-11290, ICP-11292 and ICP11294 as resistant to wilt caused by F. udum.

b-8 Embryo culture technique

Plant breeders commonly use selfing and hybridization techniques to obtain desired gene combinations. The embryos formed in interspecific and intergeneric crosses often do not survive due to the physiological barriers of incompatibility (Raghavan 1977). In such cases, hybrid embryos are aseptically separated and grown to raise hybrid

plants. The embryo rescue method is also employed to save abortion of the embryo formed (Sastri et al 1981).

The cultivated pigeonpea has many related wild species in the genus Atylosia. Some of them have desirable characters like disease and pest resistance (Ramanandam, 1980; Reddy et al 1980), which one would like to transfer to pigeonpea. In earlier studies, the rate of pod set was reported very low in most of the successful combinations and zero in several other combinations, However, the reasons are not known (Pundir 1981, Reddy et al, 1980). Further more, the use of Atylosia species in pigeonpea breeding programmes has been difficult because of problems encountered in obtaining sufficient hybrid seed in successful Cajanus-Atylosia crosses. In many such cases, hybrids have been recovered by in vitro embryo rescue technique (Stewart 1981). Distant hybridization programmes in several leguminous genera have also benefited from this technique (Braak and Koolstra 1975; Gosal and Bajaj 1983; Newell and Hymowitz 1982; Dhanju et al 1985).

By the culture of embryos, hybrids have been obtained in a number of species of Phaseolus (Honma 1955; Bajaj and Bopp 1971; Mok et al 1978, Cabral and Crocomo 1989; Munoz and Hidalgo 1986; Savova and Zagorska 1987; Andrade-Aguilar and Jackson 1988), Lens (Cohen et al 1984; Ladizinsky et al 1985), Trifolium spp. (Keim 1953; Ferguson et al 1990; Przywara et al 1989), Medicago spp. (Bauchan 1987); Glycine spp (Bognar et al 1987-88; Chung and Kim 1990), Vigna spp. (Christian et al 1987; Gosal and Bajaj 1983, Chen et al 1989), Lupinus spp. (Schafer-Menuhr et al. 1988), in the genus Arachis (Mallikarjuna and Sastri 1985; Pattee et al 1988; Stalker and Eweda 1988; Moss and Stalker 1987), Ornithopus spp. (Williams et al, 1987); etc. Belletti et al (1988) used embryos culture as a test for the screening of Phaseolus genotype.

Some cultivars of pigeonpea also have natural resistance to wilt disease, e.g. Bandapalera and NP (WR)15, but they have very low yielding potential. On the other hand, some local cultivars have high yielding potentiality, e.g. T-15-15, GAUT-82-90, but are highly susceptible to the wilt disease.

Intervarietal hybridization through conventional breeding programmes has met with little success besides being laborious and time consuming.

b-9 Ovule culture technique

Though, hybrids have been recovered by *in vitro* embryo rescue techniques in many cases (Steward 1981), embryos at very early stages of development offer greater problem of isolation and culture. In several groups of plants such as orchids, phanerogamic parasites and some saprophytes the mature embryo is so minute (Rangaswamy 1967) that its excision is extremely difficult, In *Cajanus cajan*, embryos younger than 11 days after anthesis failed to respond in culture (Kumar and Subrahmanyan 1985). The difficulty of growing very young or minute embryo led to attempts to cultures ovules. Culture of ovules is advantagous as they can be excised even at the zygote stage and are though to provide a "material environment" for the developing embryo.

Ovule culture technique has been used in several crop plants to overcome interspecific incompatability due to hybrid embryo breakdown (Cohen et al 1984, Mohapatra and Bajaj 1987, Stalker and Eweda

1988; Mallikurjuna and Sastri 1985; Moss et al 1988; Ferguson et al 1990; Przywara et al 1989).

(C) Objectives of present studies

The above discussed aspects formed the background for the present work. Considering the importance of pigeonpea to the Gujarat State in particular, and India in general, and limitation of crop yield by wilt inducing fungus, Fusarium udum, Butler, as well as very little success in evolving resistant/tolerant variety by conventional breeding programmes, we decided to address this problem using in vitro culture technology with different aims as under:

(A) Varietal screening of Cajanus cajan for wilt disease, using culture filtrate of F. udum at the whole plant and leaf disc levels;

(B) To attempt in vitro embryo rescue method for obtaining intervarietal and intergeneric viable hybrid embryos and plants;

(C) To identify the optimal cultural conditions for plantlet regeneration from various explants;

(D) To obtain a resistant line by applying selection pressure (culture filtrate of F. udum).

The results obtained in the above pursuit are incorporated under the following broad-heads:

A. Morphogenetic response from seedling explants of C. cajan (var. Bandapalera)

B. Somatic embryogenesis from cotyledons

C. Analysis of important factors and cultural manipulation for induction of somatic embryos from cotyledons

D. Field studies : 1, Intergeneric and intervarietal crosses 2, Embryo culture and ovule culture techniques

E. Pathological studies.