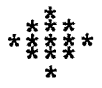
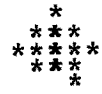



CHAPTER III

STUDIES WITH

CAJANUS CAJAN (L) MILLSP

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(A) MORPHOGENETIC RESPONSE FROM SEEDLING EXPLANTS OF CAJANUS CAJAN (L.) MILLSP.

Experiments were conducted to induce organogenesis from explants derived, from in vitro 7 days old seedlings of pigeonpea (Cajanus cajan var. Bandapalera). Various explants, viz., epicotyl, leaf and distal sections of cotyledons of 7 days old seedling of pigeonpea were examined for their ability to form (1) callus and (2) shoot regeneration. Salts of MS medium (1962) and Vitamin of B_5 medium (Gamborg's 1968) (MMS) supplemented with BAP, Kn and Ad. sulphate alone or in combinations with NAA were used for the purpose.

a-1 Callus induction and shoot regeneration from the epicotyl explants

The epicotyl explants initiated callus within five days on all media mentioned in Table A.1. Epicotyl formed slight whitish green callus at the cut ends on the 2 mg/1 BAP with 2.5 mg/1 NAA medium. Green callus was formed in 4-5 mg/l BAP alone or in combination with 0.5 mg/1 Kn and 50 mg/1 AdS medium. Epicotyl remained green during callus induction on all media tested, except when NAA was incorporated with cytokinins, it turned pale green.

After 15 days of incubation, the callus was removed and the explants were transferred to the following media: (A) MMS medium supplemented with 0.1 mg/l IBA, 0.05 mg/l GA_3, 2% Sucrose; (B) MMS medium supplemented with 0.02 mg/l Kn, 0.05 mg/l TIBA, 0.05 mg/l GA_3, 2% sucrose. Induction and proliferation of shoot buds were, in general, found to be the best with medium (A) as compared to medium (B) (Table A-1). The highest frequency of bud development was recorded when the transfer was carried out from MMS medium supplemented with BAP (5 mg/l), Kn (0.5 mg/l) and AdS (50 mg/l) to medium (A) which lacked both the cytokinins and AdS, but contained IBA and GA_3 (Fig. 1). The shoot bud development was not observed on medium (A) when transfer was carried out from MMS with NAA (2.5 mg/l) and low BAP (2.0 mg/l) levels. The treatment which recorded maximum callus induction and shoot bud development also registered the highest per cent epicotyl exhibiting response (86.7%) on medium (A). In case of medium (B), the best response with regard to callus induction, shoot and

epicotyl response was recorded when the explants were transferred from MMS supplemented with BAP (5.0 mg/l), Kn (0.5 mg/l) and AdS (50 mg/l) or with MMS with NAA (2.5 mg/l), BAP (5.0 mg/l), Kn (0.5 mg/l) and AdS (50 mg/l).

It was found that the addition of NAA (2.5 mg/l) in the induction medium suppressed epicotyl showing the same degree of response on (A) medium. For example, induction media without NAA registered 66 and 86.7% epicotyl exhibiting response on medium (A) as compared to 50 and 56% respectively, with induction media containing 2.5 mg/l NAA. On the other hand, on medium (B), in general, no significant suppression in epicotyl response was observed with NAA containing induction medium. Overall, the epicotyl response was considerably less on medium (B) as compared to medium (A).

When the shoot buds developed upto 2 to 3 cm tall, they were excised and transferred on half strength MMS medium supplemented with 0.1 mg/l IBA and 0.05 mg/l GA_3 and incubated for 7 days for root induction. Root primordia differentiated within a week of inoculation

Table A.1 : Shoot regeneration from epicotyl explants of 7 days old seedlings of C. cajan (var. Bandapalera)

Sr. No.	Induction media	Regeneration media					
		A			B		
		Degree of response		Epicotyl forming shoot (%)	Degree of response		Epicotyl forming shoot (%)
		Frequency of recallusing	Frequency of shoot formation		Frequency of recallusing	Frequency of shoot formation	
1	MMS + 2 mg/l BAP	+ +	+	66 (±11.7)	+	+	30 (± 8.1)
2	MMS + 2.5 mg/l NAA + 2 mg/l BAP	+	+	50 (±10.0)	+	+	40 (±16.3)
3	MMS + 4 mg/l BAP	+ + + +	+	60 (±14.1)	+ +	+	25 (± 4.7)
4	MMS + 2.5 mg/l NAA + 4 mg/l BAP	+ +	+	56 (±10.8)	+ +	+	30 (± 3.7)
5	MMS + 5 mg/l BAP + 0.5 mg/l Kn + 50 mg/l AdS	+ + + +	+ + +	86.7(± 8.2)	+ +	+ +	62.5(±12.5)
6	MMS + 2.5 mg/l NAA + 5 mg/l BAP + 0.5 mg/l Kn + 50 mg/l AdS	+ +	+	60 (±16.3)	+	+ +	60 (±14.1)

A = MMS + 0.1 mg/l IBA + 0.05 mg/l GA_3

B = MMS + 0.02 mg/l Kn + 0.5 mg/l TIBA + 0.05 mg/l GA_3

Value in parenthesis is ± SD

Value		
-	Nil	Nil No. of shoot
+	Low	0 - 5 "
++	Moderate	5 - 10 "
+++	High	10 - 15 "
++++	Very high	

on rooting medium (Fig. 2). Shoots alongwith differentiated root primordia were transferred on half strength MMS basal medium for further growth. The elongation of the roots was found more rapid than shoot development (Fig. 3).

a-2 Shoot regeneration from seedling leaf explants

On MMS medium with different growth regulators, either singly or in different combinations, leaf explants of seedlings exhibited direct shoot regeneration accompanied by varying amounts of callus formation. The leaf expansion was maximum on the induction medium containing high levels of cytokinins. When NAA was incorporated with cytokinins in the induction medium, a depression in the response with the formation of callus at the cut ends of the leaf was observed which was pale green in colour.

Explants on induction medium were transferred after 15 days of incubation to shoot regeneration medium supplemented with IBA (0.1 mg/l) and GA_3 (0.05 mg/l) (Medium A) or TIBA (0.5 mg/l), Kn (0.02 mg/l) and GA_3 (0.05 mg/l) containing medium (B). After one

Fig. 1 Shoot regeneration from epicotyl explants of 7 days old seedling (Var. Bandapalera)

Age of culture : 25 days

Medium : MMS + 0.1 mg/l IBA + 0.05 mg/l GA_3

Fig. 2 Root induction from regenerated shoot of var. Bandapalera after 7 days incubation

Medium : ½ MMS + 0.1 mg/l IBA + 0.05 mg/l GA_3

X2.0

Fig. 3 Complete plantlet from epicotyl of 7 days old seedling (var. Bandapalera) six week old

1

2

3

month, either direct shoot development was noted from leaf perifery or with slight callusing only on medium (B) in explants transferred from induction media containing high level of cytokinins and adenine sulphate with or without NAA. Leaf expansion was more or less similar on both the regeneration media, but high cytokinins and adenine sulphate with or without NAA in induction medium registered higher leaf expansion. In case or regeneration medium (A), in general, incorporation of NAA in induction medium suppressed leaf expansion, except with high cytokinins medium where no such suppression was noted in leaf expansion (Table A-2).

Explants turned yellowish or palegreen in colour on both the regeneration media when transferred from induction media containing low or moderate level of cytokinins (2-4 mg/l BAP) alone or with NAA (2.5 mg/l). Recallusing was less on regeneration medium (A) as compared to medium (B). Overall shoot development was the highest when explants from MMS with

Table A-2 : Shoot regeneration from leaf explants of 7 days old seedlings of C. cajan (var. Bandapalera)

Sr. No.	Induction media	Regeneration media							
		A				B			
		Degree of response				Degree of response			
		Frequency of shoot induction	Frequency of recallusing	Expansion of leaf	Colour development of leaf	Frequency of shoot induction	Frequency of recallusing	Expansion of leaf	Colour development of leaf
1	MMS + 2 mg/l BAP	-	+	+ +	+ +	-	+	+ +	+
2	MMS + 2.5 mg/l NAA + 2mg/1 BAP	-	+	+	+	-	+ +	+ +	+
3	MMS + 4 mg/l BAP	-	-	+ + +	+ +	-	-	+ +	+
4	MMS + 2.5 mg/l NAA + 4 mg/l BAP	-	+	+ +	+	-	+	+	+
5	MMS + 5 mg/l BAP + 0.5 mg/l Kn + 50 mg/l AdS	-	+	+ + +	+ + +	+ +	+ +	+ + +	+ + +
6	MMS + 2.5 mg/l NAA + 5 mg/l BAP + 0.5 mg/l kn + 50 mg/l AdS	-	+	+ + +	+ +	+	+ + +	+ + +	+ +

Value of degree	Shoot induction	Callus induction	Leaf expansion	Colour development
-	Nil	Nil	-	-
+	0 - 5	Low	Low	Yellowish green
+ +	5 - 10	Moderate	Moderate	Pale green
+ + +	10 - more	Highe.·	Full	Dark green

cytokinins and adenine sulphate were transferred to the shoot regeneration medium (B) (Fig. 4).

The regenerated shoots were transferred to half strength MMS medium, supplemented with 0.1 mg/l IBA and 0.5 mg/l GA_3 for root induction. Root initiation was observed within a week of incubation. These shoots with root initials were transferred on to half strength MMS basal medium for further growth. Root growth was found to be quite fast (Fig. 5).

a-3 Induction of embryos from cotyledons

The distal halves of the cotyledons (from 7 days old seedling) expanded rapidly and turned green within ten days on induction medium having cytokinins alone. When NAA was incorporated in the medium with cytokinins, cotyledons expanded rapidly but turned pale green. More amount of whitish green compact callus was initiated from cut ends, within ten days of inoculation on induction medium supplemented with a 4 mg/l BAP as compared to other treatments. Embryoids were differentiated within twenty days on induction medium containing

Fig. 4 Shoot regeneration from leaf explants of 7 days old seedling (var. Bandapalera)

Age of culture : One month

Medium : MMS + 0.5 mg/l TIBA + 0.02 mg/l Kn + 0.05 mg/l GA_3

X1.3

Fig. 5 Rooted plantlet from leaf explants (var. Bandapalera), 7 weeks old culture

4

1
2
3
4
5
6
7
5

high levels of cytokinins (5 mg/l BAP, 0.5 mg/l Kn and 50 mg/l AdS). Embryoid differentiation was not observed on any other inductioon media within 20 days of incubation.

After 20 days of incubation on induction medium, the explants were transferred (after removal of callus) to regeneration medium (A) containing IBA (0.1 mg/l) and GA_3 (0.05 mg/l) or regeneration medium (B) containing Kn (0.02 mg/l), TIBA (0.5 mg/l) and GA_3 (0.05 mg/l). Recallusing was observed on both the regenration media, except explants transferred from induction medium containing cytokinins (BAP 5 mg/l and Kn 0.5 mg/l), NAA (2.5 mg/l) and AdS (50 mg/l0 to regeneration medium (A) and from induction medium containing BAP (2.0 mg/l) to regeneration medium (B). Frequency of embryo formation was maximum on regeneration medium (A) in explants transferred from induction medium containing 5 mg/l BAP, 0.5 mg/l Kn and 50 mg/l AdS (Table A-3, Fig. 6). The embryoid formation was not observed on regeneration medium (B), when explants were transferred from induction medium containing

Table A-3 : Embryo induction from cotyledon explants of 7 days old seedling of C. cajan (var. Bandapalera)

Sr. No.	Induction media	Regeneration media					
		A			B		
		Degree of response			Degree of response		
		Frequency of recallus-ing	Frequency of embryo induction	Cotyledons (%) with embryo induction	Frequency of recallus-ing	Frequency of embryo induction	Cotyledons (%) with embryo induction
1	MMS + 2 mg/l BAP	+	+	80 (± 14.1)	-	+	25 (± 6.3)
2	MMS + 2.5 mg/l NAA + 2 mg/l BAP	+ +	+	80 (± 16.3)	+	+	40 (± 11.7)
3	MMS + 4 mg/l BAP	+ +	+	80 (± 16.3)	+	+	50 (± 7.0)
4	MMS + 2.5 mg/l NAA + 4 mg/l BAP	+ +	+	75 (± 17.6)	+ +	+	60 (± 16.3)
5	MMS + 5 mg/l BAP + 0.5 mg/l Kn 50 mg/l AS	+	+ + +	100 (± 0)	+	+	70 (± 8.2)
6	MMS + 2.5 mg/l NAA + 5 mg/l BAP + 0.5 mg/l Kn + 50 mg/l AS	-	+ +	85 (± 9.4)	+ +	+	70 (± 10.0)

Regeneration media (A) MMS + 0.1 IBA + 0.05 mg/l GA_3

(B) MMS = 0.02 mg/l Kn + 0.5 mg/l TIBA + 0.05 mg/l GA_3

Value in parenthesis is SD

Value

- \- Nil
- \+ Low
- ++ Moderate
- +++ High

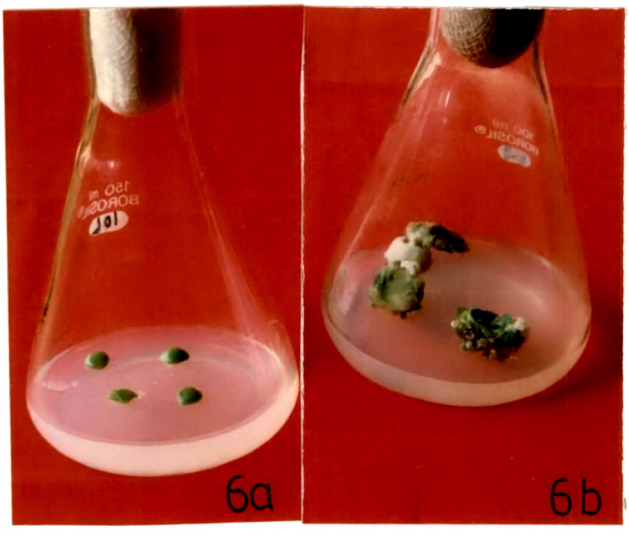
Fig. 6 Embryo induction from cotyledon explants of 7 days old seeding (var. Bandapalera)

a. The distal halves of cotyledon (initial)

b. Embryo induction after 20-25 days

Medium : MMS + 5 mg/l BAP + 0.5 mg/l Kn
+ 50 mg/l AdS

X1.8



6a 6b

low level of cytokinins (2 mg/l BAP) with or without NAA (2.5 mg/l). On the whole, the embryoid frequency as well as embryoid inductive cotyledons were less on regeneration medium (B) as compared to medium (A). Explants transferred from inductive medium containing cytokinins (BAP and Kn) and adenine sulphate onto regeneration medium (A) exhibited 100% embryoid induction from the cotyledonary explants.

(B) SOMATIC EMBRYOGENESIS FROM COTYLEDONS

b-1 Developing simple protocol for somatic embryogenesis

In earlier experiments plant regeneration through organogenesis from different explants of aseptic seedling of C. cajan has been described. However, the frequency of regeneration through organogenesis was low. Plant regeneration was also tried by another approach, i.e. somatic embryogenesis as given below:

Somatic embryogenesis

The sterilized seeds of C. cajan, var. Bandapalera were incubated in sterile water on gyrotary shaker for 16 h. After incubation period was over, the seeds were removed and the cotyledons detached from the embryo axis.

The cotyledons were cut transversely and only the distal halves of cotyledons were cultured on different media.

Induction

The distal halves of the cotyledons expanded rapidly and turned green after two weeks of incubation on MMS supplemented with 5.0 mg/l BAP, 0.5 mg/l Kn and 50.0 mg/l AdS (medium 5 in Tables A-2 and A-3). The same medium was found to be the most responsive medium in earlier experiments on organogenesis. Proliferation of somatic embryos was observed on both adaxial and abaxial surfaces of the cotyledon explants on this medium along with compact whitish green callus from the cut ends (Fig. 7).

Maturation

However, the embryos did not develop further on medium 5. The explants from medium 5 were, therefore, transferred to the MMS medium containing one tenth of all the hormonal supplements of medium 5 (medium C). Further development of embryos was observed on this medium and as the embryos matured, more and more embryos differentiated on this medium

(Fig. 8). Different stages of embryo development (globular, heart and torpedo shaped) were observed on this medium (Fig. 9 to 12). Slight callusing from cut ends was also noticed.

Germination

For germination, the embryos from medium C were transferred to medium D, which contained MMS basal medium supplemented with 0.1 mg/l IBA and 0.05 mg/l GA_3. The mature embryos germinated and developed into complete plantlets within 25-30 days on medium (D) (Fig. 13 to 15). At the same time, many secondary embryos were also formed. Thus, the number of embryos were makredly multiplied on both C and D media. The germinated embryos (plantlets) were transferred for root and shoot growth on one tenth strength of medium (D).

Different stages of embryo development were confirmed by histological examination (Fig. 16, A-D).

(C) ANALYSIS OF IMPORTANT FACTORS AND CULTURAL MANIPULATIONS FOR INDUCTION OF SOMATIC EMBRYOS FROM COTYLEDONS

The effect of few important factors together with cultural manipulations on somatic embryogenesis from cotyledons is analysed in this section.

Fig. 7 Induction of somatic embryos on the distal halves the cotyledon (var. Bandapalera)

Age of culture : Two week

Medium : MMS + 5 mg/l BAP + 0.5 mg/l Kn + 50 mg/l AdS

Fig. 8 A cluster of globular, heart and torpedo shaped embryos developed on surface of cotyledon (var. Bandapalera) after 2-3 week on maturation medium C.

"C" - Medium : MMS + 0.5 mg/l BAP + 0.05 mg/l Kn + 5 mg/l AdS

X 22

Fig. 9 The same under higher magnificant

X 40

Fig. 10 Isolated globular embryo

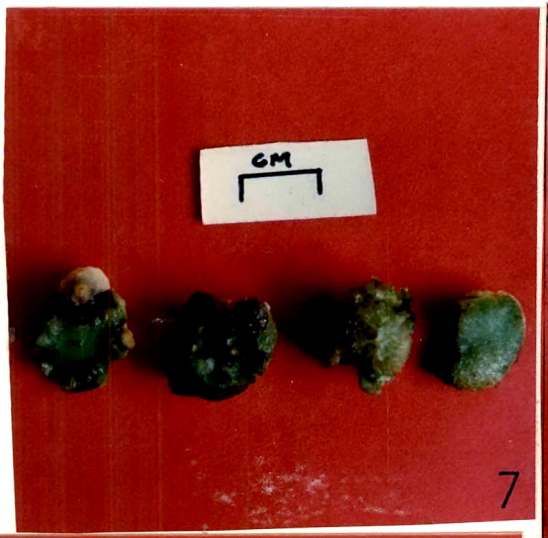
X100

Fig. 11 Isolated heart shaped emrbyo

X100

Fig. 12 Isolated torpedo shaped (t) and cotyledonary (c) embryos

X65



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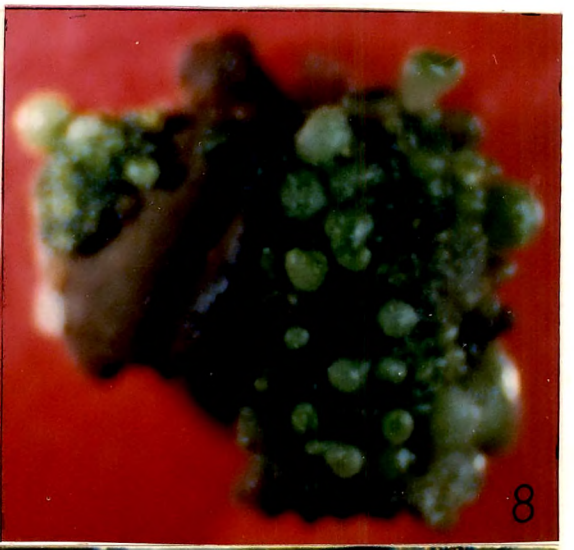
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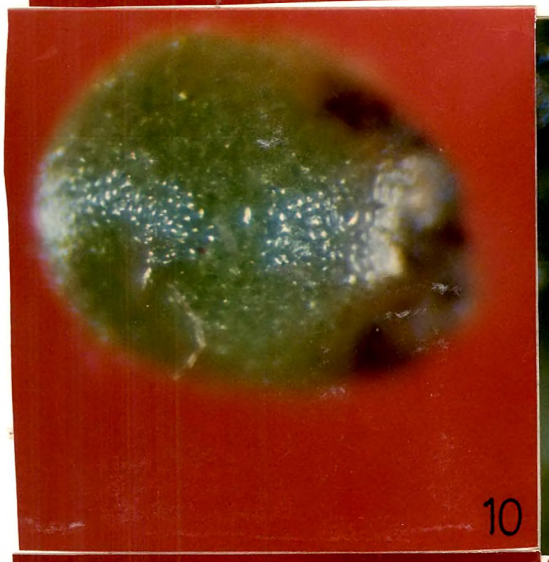
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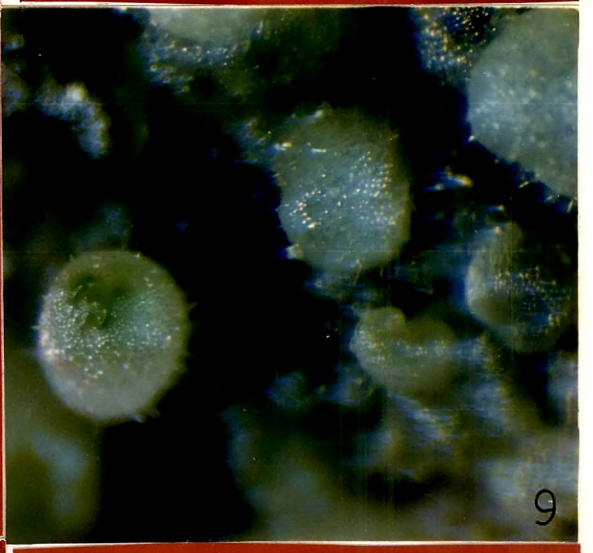
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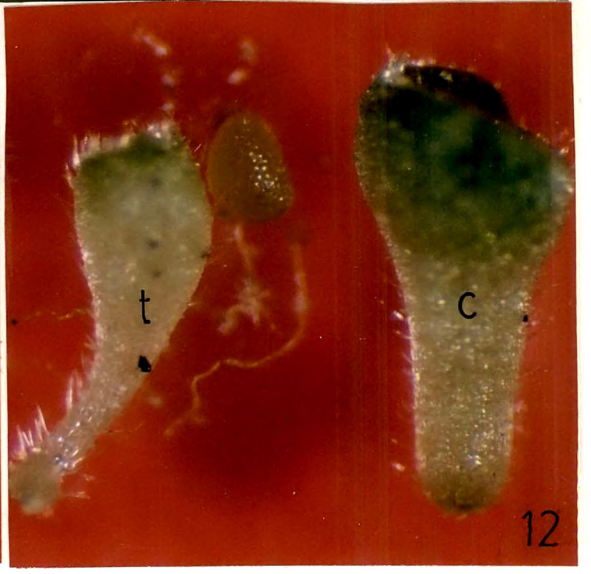

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Fig. 13 Germination of embryos after 2 weeks and formation of secondary embryos (arrow marked)

medium "D": mmS + 0.5 mg/l IBA + 1 mg/l GA_3

X1.5

Fig. 14 Germination of somatic embryos with defined cotyledons and root in the same medium.

X2.5

Fig. 15 Regeneration of complete plantlet (var. Bandapalera) with well developed root and shoot systems from somatic embryo after 45-50 days

X2.5


13


14


15

Fig. 16

A. A group of somatic embryos in different developmental stages

X40

B. L. S. of somatic embryo showing root (rt) portion

X220

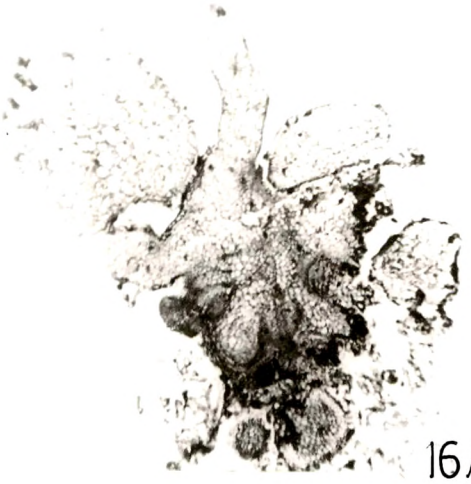
C. L. S. of cotyledon showing induction of somatic embryos in a row, in 15 days old culture

X140

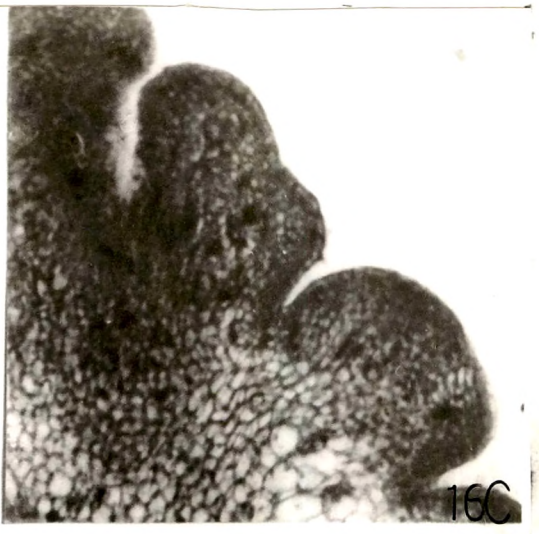
D. L. S. of somatic heart shaped embryo.

X140

Medium; MMS + 0.5 mg/l BAP + 0.05 mg/l Kn + 5 mg/l AdS



16A



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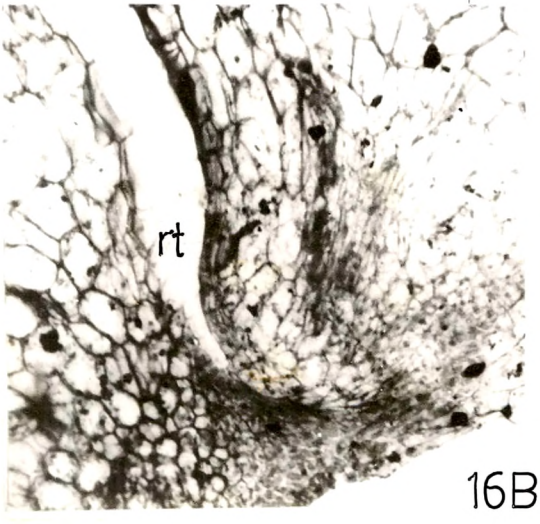
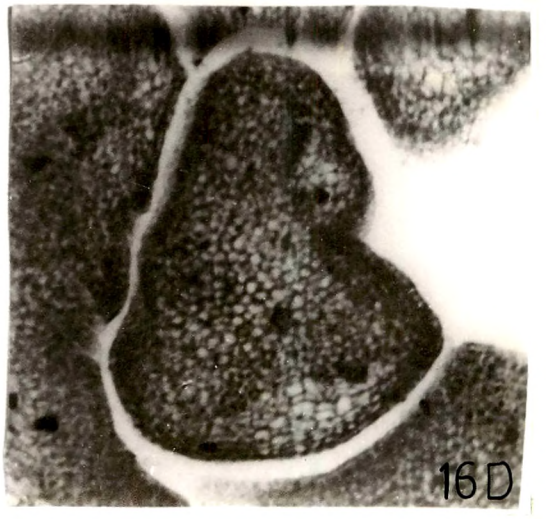


16B



16D

c-1 Effects of auxins and cytokinins on embryo induction

The effect of cytokinins and auxins on embryo induction was examined by culturing distal halves of cotyledons (C. cajan var. Bandapalera) on MMS basal medium supplemented with different levels of cytokinins (BAP and Kn) and/or auxin (NAA) (Table C-1).

The hormonal supplements markedly affected the frequency of embryo induction and the number of responsive cotyledons. Upto 50% of cotyledons responded on MMS medium containing low levels of cytokinins (1.125 to 2.5 mg/l BAP and 0.5 mg/l Kn) alone or in combination with low levels of NAA (0.5 to 1.0 mg/l). On the other hand, eighty per cent or more cotyledons responded in presence of high level of cytokinin (5.0 mg/l BAP) alone or along with 0.5 mg/l or 2.5 mg/l NAA. The highest frequency of embryo induction was registered on medium containing high cytokins (5.0 mg/l BAp and 0.5 mg/l Kn) followed by medium with high BAP (5.0 mg/l) with 2.5 mg/l NAA. Incorporation of low levels (0.5 to 1.0 mg/l) of NAA suppressed embryo induction. Thus, the frequency of embryo

Table C-1 : Effect of NAA with different levels of cotykinins on embryo induction from cotyledon explants of *C. cajan* (var. Bandapalera)

	Induction media	Degree of response		Responsive cotyledon (%)
		Frequency of callus induction	Frequency of embryo induction	
1.	MMS + 1.125 mg/l BAP + 0.5 mg/l Kn + 50 mg/l AS	-	+	10 (± 0)
2.	MMS + 2.5 mg/l BAP + 0.05 mg/l Kn 50 mg/l AS	+	+	10 (± 0)
3.	MMS + 5 mg/l BAP + 0.5 mg/l Kn + 50 mg/l AS	+ +	+ + + +	90 (±10)
4.	MMS + 0.5 mg/l NAA + 2.5 mg/l BAP 0.5 mg/l Kn + 50 mg/l AS	+	+	4.6(± 4.7)
5.	MMS + 0.5 mg/l NAA + 5 mg/l BAP + 0.5 mg/l Kn + 50 mg/l AS	+	+ +	80 (±16.3)
6.	MMS + 1.0 mg/l NAA + 2.5 mg/l BAP + 0.5 mg/l Kn + 50 mg/l AS	+	+	50 (± 0)
7.	MMS + 1.0 mg/l NAA + 5 mg/l BAP + 0.5 mg/1 Kn + 50 mg/l AS	+	+	60 (±14.1)
8.	MMS + 2.5 mg/l NAA + 2.5 mg/l BAP + 0.5 mg/l Kn + 50 mg/l AS	+ +	+ +	61.5(±10.2)
9.	MMS + 2.5 mg/l NAA + 5 mg/l BAP + 0.5 mg/l Kn + 50 mg/l AS	-	+ + +	84.6(10.8)
10.	MMS + 5 mg/l NAA + 2.5 mg/l BAP + 0.5 mg/l Kn + 50 mg/l AS	+	+ +	75.0(±20.4)
11.	MMS + 5 mg/l NAA + 5 mg/l BAP + 0.5 mg/l Kn + 50 mg/l AS	+	+ +	76.9(± 2.3)
12.	MMS + 10 mg/l NAA + 2.5 mg/l BAP + 0.5 mg/l Kn + 50 mg/l AS	+	+	60.0(±16.3)
13.	MMS + 10 mg/l NAA + 5 mg/l BAP + 0.5 mg/l Kn + 50 mg/l AS	+	+ +	75.0(±17.6)
14.	MMS + 20 mg/l NAA + 2.5 mg/l BAP + 0.5 mg/l Kn+50 mg/l AS	+	+	60.0(±16.3)
15.	MMS + 20 mg/l NAA + 5 mg/l BAP + 0.5 mg/l Kn + 50 mg/l AS	+	+	64.7(±10.6)

Value :-

\- Nil, + Low, ++ MOderate, +++ High, ++++ Higher

induction or responsive cotyledons per cent were, in general, limited or suppressed on media containing low levels of NAA or low level of cytokinins. The callus induction was observed in most of the treatments, except in the presence of low cytokinins and high cytokinin with 2.5 mg/l NAA treatments. The former treatment also recorded very low per cent of responsive cotyledons as well as low frequency of embryo induction. However, the later treatment exhibited 84.6% responsive cotyledons and considerably high frequency of embryo induction.

c-2 Influence of imbibition period on embryo induction from cotyledons

The sterilized seeds of C. cajan, var. Bandapalera were imbibed in sterile water on a gyrotary shaker for different periods of time (8, 12, 16, 20, 24, and 36 h). The distal halves of cotyledons were then excised and cultured on MMS medium supplemented with 5 mg/l BAP, 0.5 kg/k Kn and 50 mg/l AdS (induction medium). The observations on cotyledon response, embryo induction frequency and callus induction

were recorded after 20-25 days of incubation on induction medium (Table C-2).

The imbibition period of seed showed distinct effect on the embryo induction frequency, per cent responsive cotyledons and callus formation from cut ends of cotyledons. It was observed that cotyledons of seeds imbibed for 16 and 20 h expanded rapidly and turned pale or dark green in colour on induction medium. Increasing imbibition period beyond 20 h increased callus formation from the cut ends of cotyledon explants. The peak in the embryo induction frequency and per cent responsive cotyledons (84.2 to 85.7%) was recorded with seeds imbibed for 20 and 16 h. There was a sharp decline in embryo induction frequency as well as responsive cotyledons when imbibition was less than 16 h. However, the decline was less when compared with imbibition for more than 20 h. On the other hand, callus induction was minimum in cotyledons obtained from seeds imbibed for 16 to 20 h.

c-3- Effect of imbibition in BAP on embryo induction

In an another set of experiments, the seeds of C. cajan var. Bandapalera were imbibed in

Table C-2 : Effect of imbibition period on embryo induction from cotyledonex plants of C. cajan, var. Band palera

Imbibation periods	Response of cotyledons (%)	Frequency of embryo induction	Frequency of callus induction
8 h	44.4	+	+ +
12 h	60.0	+	+ +
16 h	85.7	+ + +	+
20 h	84.2	+ + +	+
24 h	75.0	+ +	+ + +
36 h	68.7	+ +	+ + +

Induction medium : MMS + 5 mg/1 BAP + 0.5 mg/l Kn + 50 mg/l AdS.

\+ = Low

++ = Moderate

+++ = High

different concentrtion of BAP for 16 h. The cotyledons excised from imbibed seeds were cultured on induction medium as reported in earlier section c-2.

There was decrease in embryo induction frequency as well as induction per cent of embryo from cotyledons in BAP imbibed seeds; increasing concentration of BAP was found to have more negative effect. The highest embryo inductive frequency and embryo inductive cotyledons were recorded in the control cotyledons obtained from seeds imbibed in sterile water. On the other hand, pronounced increase in callus induction was registered in cotyledons with BAP imbibition treatment; the highest level of BAP tested (1.0 mg/l) was found to promote the maximum amount of callus formation (Table C-3).

c-4 Influence of salt formulation on embryo induction from cotyledons of different C. cajan genotypes

The distal halves of cotyledons obtained from four different genotypes [T-15-15, CAUT-82-90, Bandapalera, NP (WR) 15] of C. cajan were cultured separately on various salt formulations (media), each supplemented with 5 mg/l BAP, 0.5 mg/l Kn

Table C-3 : Effect of imbibition in BAP solution on embryo induction from cotyledon explants of C. cajan (var. Bandapalera)

(Soaking for 16 h)

Soaking treatment*	Response of		Degree of response	
	Embryo forming coty-ledons (%)	Callus forming coty-ledons 95)	Frequency of embryo induction	Frequency of callus induction
Water	70.8	16.6	+ + +	+
BAP 0.1 PPM	63.6	22.7	+ +	+
BAP 0.5 PPM	60.0	20.0	+	+ +
BAP 1.0 PPM	46.4	26.6	+	+ +

Induction medium : MMS + 5 mg/l BAP + 0.5 mg/l KN + 50 mg/l AdS

Value

\+ = Low

++ = Moderate

+++ = High

and 50 mg/l AdS. The percentage of responsive cotyledons, frequency of embryo induction, fresh and dry wts per cotyledon on each medium for all the four genotypes tested are given in Table C-4. These observations were recorded after 20 to 25 days incubation on each medium.

Of the genotypes tested, NP (WR) 15 showed maximum number of responsive cotyledons, followed by genotypes Bandapalera, GAUT 82-90 and T-15-15 on the media tested. The maximum frequency of embryo induction was observed in the cotyledons of variety Bandapalera and minimum in the variety NP (WR) 15. On medium EC-6, the per cent frequency of embryo induction was highest in all the varieties, except var. GAUT-82-90 were maximum embryogenesis was recorded on medium MMS (Table C-4). However, healthy embryos capable of producing green leafy shoots were obtained only on MMS medium in all the varieties. On B_5 and MB_5 formulations, they became viterous, whereas on EC_6, LS and white's, they turned chlorotic and failed to develop beyond the late heart to early cotyledon stages. The per cent response cotyledons was maximum on MMS medium in var. T-15-15, GAUT-82-90 as well as NP (WR) 15;

Table C-4: Effect of salt formulations on embryo induction from cotyledons explants of different pigeonpea varieties

Variety	T-15-15				GAUT-82-90				Bandapalera				NP (WR) 15			
Salt formulation	Responsive cotyledons %	Frequency of embryo induction %	Av. fresh wt/ coty. mg	Av. dry wt/ coty mg	Responsive coty. %	Frequency of embryos induction %	Av. fresh wt/ coty. mg	Av. dry wt/ coty mg	Responsive coty. %	Frequency of embryo induction %	Av. fresh wt/ coty. mg	Av. dry wt/ coty. mg	Responsive coty %	Frequency of embryo induction %	Av. fresh wt/ coty mg	Av. dry wt/ coty. mg
MMS	80.0 (±11.0)	64.3 (±28.5)	608.7 (±327.7)	55.1 (±18.6)	85.0 (±.20.0)	83.0 (±42.4)	559.4 (±218.3)	48.3 (±13.9)	70.0 (±.18.7)	96.5 (±24.9)	518.4 (±167.7)	50.4 (±11.2)	100.0 (± 0)	64.0 (±12.2)	292.1 (±90.5)	28.1 (±6.7)
EC-6	55.0 (±18.7)	81.8 (±38.2)	312.2 (49.4)	37.8 (±5.5)	60.0 (±20.0)	68.3 (±36.9)	380.6 (±143.3)	38.8 (10.8)	65.0 (±20.0)	98.4 (±38.2)	715.7 (±202.1)	55.1 (±12.4)	100.0 (± 0)	81.0 (±22.6)	457.1 (±193.5)	36.9 (±11.5)
LS	60.0 (±20.0)	80.0 (±46.1)	292.0 (±67.4)	41.6 (±5.9)	65.0 (±20.0)	53.8 (±26.4)	375.5 (±114.6)	39.2 (±11.2)	85.0 (±20.0)	92.6 (±17.6)	440.3 (±141.8)	44.2 (±11.6)	100.0 (± 0)	80.0 (±26.4)	408.7 (±182.7)	35.9 (±9.6)
W	60.0 (±12.1)	54.5 (±29.6)	266.6 (±45.4)	37.7 (±6.2)	85.0 (±20.0)	47.0 (±29.0)	266.3 (±33.5)	32.5 (±3.1)	90.0 (±12.2)	82.0 (±20.0)	302.8 (±43.2)	38.4 (±4.7)	95.0 (±10.0)	14.0 (±5.5)	232.2 (±46.33)	28.2 (±4.9)
B_5	55.0 (±10.0)	50.0 (±17.8)	291.6 (±34.0)	37.0 (±6.0)	55.0 (±24.4)	60.0 (±30.0)	331.0 (±56.9)	37.2 (±5.7)	60.0 (±20.0)	84.0 (±27.0)	599.5 (±245.2)	51.2 (±109.0)	90.0 (±10.0)	48.0 (±12.2)	500.4 (±212.7)	39.9 (±14.0)
MB_5	50.0 (±17.6)	35.0 (±15.0)	276.6 (±57.6)	36.2 (±8.6)	45.0 (±24.4)	60.0 (±29.8)	394.6 (±134.6)	40.0 (±7.6)	75.0 (15.8)	56.0 (±24.4)	375.5 (±135.1)	35.1 (±7.1)	95.0 (±8.6)	63.0 (±20.5)	426.4 (±168.9)	33.6 (±8.0)

Value in parentheses is SD.

whereas in var. Bandapalera it was registered on white's medium. Var. GAUT-82-90 also recorded highest responsive cotyledons on white's medium. Similarly, medium EC-6 and LS evoked maximum response from cotyledons in Var. NP (WR) 15.

In var. T-15-15 and GAUT-82-90, MMS formulation supported maximum fresh and dry weights of cotyledons; whereas it was on EC-6 formulation in var. Bandapalera and on B_5 in var. NP (WR) 15.

c-5 Influence of age and size of immature cotyledons on embryo induction

This experiment was performed to examine the response of age and size of immature cotyledons for embryo induction on inductive media i.e. MMS medium supplemented with 5.0 mg/l BAP, 0.5 mg/l Kn and 50 mg/l AdS. Excised distal halves of cotyledons from sterile pods of C. cajan var. T-15-15 were cultured on induction medium for a period of 15-20 days. The observations on growth of cotyledons in terms of fresh and dry weights, degree of cotyledon expansion and callus induction, per cent cotyledons response, cotyledons (%) forming

embryos and frequency of embryo induction are presented in Table C-5.

It is evident from the data that there was a clear effect of age and size of immature cotyledons on embryo induction. The very young (15 days old) and small size (3 mm x 4 mm) cotyledons failed to give any response; whereas oldest (35 days) and biggest (7 mm x 9 mm) registered high frequency of embryo induction as well as highest (83.8%) per cent responsive cotyledons for embryo induction. In general, increasing response was noted with increase in size and age of the cotyledons. The cotyledons of 35 days age and 7 mmm x 9 mm size also recorded highest degree of cotyledon expansion and callus induction, cent per cent responsive cotyledons and high growth of cotyledons.

c-6 Effect of amino acids, ABA, $AgNO_3$ and salicylic acid on maturation and germination of embryos

The importance of reduced nitrogen and certain amino acids and amides (Ammirato 1983 b) has continued to be reaffirmed. For example, glutamine and asparagine were important for maturation and germination of somatic embryos

Table C-5 : Influence of age and size of immature cotyledons on embryos induction (var. T-15-15)

Age of immature cotyledon (days after anthesis)	Size of coty-ledon (mm)	Initial fresh and dry wts. of cotyledon		% Respon-sive coty-ledon	Degree of callus induc-tion	Cotyoedons forming embryos %	Frequ-ency of embryo induc-tion	Degree of coty-ledon expan-sion	Fresh & Dry wt. of one cotyledon after 15-20 days incubation	
		Fresh wt. mg/coty.	Dry wt. mg/coty.						Fresh wt. mg/coty	Dry wt. mg/coty
A 15 days	3 x 4	3.5 (± 0.7)	0.8 (±0.17)	0.0	0.0	0.0	0.0	0.0	3.0 (±0.06)	0.6 (±0.15)
B 20 days	4 x 5	13.8 (± 3.8)	2.9 (±1.08)	54.2 (±14.8)	+	26.0 (±17.8)	+	++	109.8(±61.2)	11.8 (±5.10)
C 20 days	5 x 6	35.8 (± 5.9)	8.1 (±2.17)	84.1 (±18.6)	+	36.2 (±15.2)	++	++	177.0(±55.6)	20.1(± 3.5)
D 30 days	6 x 7	64.1 (± 9.7)	22.0 (±6.00)	89.3 (±12.3)	++	68.2 (±20.8)	+++	+++	351.8(±150.3)	31.5(± 8.9)
E 35 days	7 x 9	87.0 (±13.7)	40.6 (± 0.00)	100.0 (± 0.0)	+++	83.3 (±11.8)	++++	++++	475.6(±229.3)	62.5(±23.7)

Induction medium : MMS + 5.0 mg/l BAP + 0.5 mg/l Kn + 50 mg/l Ad. sulphate + 2 % sucrose

Degree of callus or frequency of embryos

0 Nil
\+ Low
++ Moderate
+++ High
++++ Very high

in soybean (Obsendorf and Slawinska 1988) and in Narway spruce (Boulay et al 1988). Similarly, ABA also benefited somatic embryo maturation and germination in soybean (Ranch et al 1985, Ghazi et al 1986). Ethelene typically inhibits somatic embryogenesis (Ammirato 1983 b). A tryptophan analogue, 5 methyl tryptophan is also reported to promote high degree of embryo maturation in soapnut (Desai et al 1986).

In view of these findings, the effect of two amino acids viz., glutamine and asparagine as well as ABA, $AgNO_3$ and salicylic acid on maturation and germination of C. cajan was examined in the present investigation.

The distal halves of the immature cotyledons of C. cajan var. T-15-15 were inoculated on induction medium (MMS + 5 mg/l BAP + 0.5 mg/l Kn + 50 mg/l AdS). After 20 days the green swollen embryogenic cotyledons were transferred onto the same medium but with ten fold reduction in hormonal concentrations, i.e. 0.5 mg/l BAP + 0.05 mg/l Kn + 5 mg/l AdS and further supplemented with two different amino acids with or without ABA, $Agn0_3$ or salicylic acid

to test their effect on maturation and germination of embryos.

After 20-25 days on the medium containing L-glutamine or L-asparagine with or without ABA, per cent maturation (30-45%) and intensity of secondary embryo formation were low as compared to the control (MMS + 0.5 mg/l BAP + 0.05 mg/l Kn + 5 mg/l AdS). Moreover, most of the embryos failed to undergo further development and recallused. Similarly, ABA and salicylic acid treatments also failed to enahnce embryo maturation and secondary embryo formation as compared with the control (Table C-6). On the other hand, $AgNO_3$ supplemented at 20 mg/l supported maximum embryo maturation and germination (75 to 80%) as well as secondary embryo formation. Many secondary embryos in different stages of development were observed. Some healthy embryos capable of producing green leafy shoots were obtained on this medium.

(D) FIELD STUDIES

The present study attempts to apply embryo rescue technique to Cajanus x Atylosia hybrid embryos, because of the difficulties encountered

Table C-6 : Effect of amino acids, salicylic acid, ABA and $AgNO_3$ on embryo maturation, germination and formation of secondary embryos in C. cajan, var. T-15-15

Media	Intensity of secondary embryo formation	Embryo maturation and germination (%)
MMS + 0.5 mg/l BAP ± 0.05 mg/l Kn + 5 mg/l AdS + 4% sucrose (Control)	+++	55 - 60
C + 0.025 mg/l ABA	++	45 - 50
C + 0.25 mg/l ABA	+	35 - 40
C + 20 mg/l $AgNO_3$	++++	75 - 80
C + 40 mg/l $AgNO_3$	+++	55 - 60
C + 200 mg/l L-Asparagine	++	35 - 40
C + 200 mg/l L-Asparagine + 0.025 mg/l ABA	+	35 - 40
C + 200 mg/l L-Asparagine + 0.25 mg/l ABA	+	40 - 45
C + 200 mg/l L-Glutamine	++	30 - 35
C + 200 mg/l L-Glutamine + 0.025 mg/l ABA	++	35 - 40
C + 200 mg/l L-Glutamine + 0.25 mg/l ABA	+	25 - 30
C + 100 mg/l salicylic acid	+++	55 - 60
C + 200 mg/l salicylic acid	++	40 - 45

Degree of embryo formation

+ Low
++ Moderate
+++ High
++++ Very high

in obtaining successful Cajanus x Atylosia crosses reported by Reddy et al (1980) and Pundir (1981). Similarly attempts for embryo rescue method were also made for obtaining viable embryos and plants from crosses of susceptible x resistant natural cultivars of pigeonpea because of low success in intervarietal hybridization through conventional breeding programmes.

1. Intergeneric crosses

Crosses were made between Atylosia lineata and C. cajan var. T-15-15 and GAUT-82-90 on different dates as and when flowers of A. lineata were available during May 1989 using T-15-15 and GAUT-82-90 as female parents. In both the cases crosses were unsuccessful (Table D-1). Though A. lineata cross with var. T-15-15 yielded two pods, seed setting was not obtained in the pods.

2. Intervarietal crosses

The natural wilt resistant cultivars NP (WR) 15 and Bandapalera of C. cajan were employed as the male parents in the crosses with susceptible cultivars T-15-15 and GAUT-82-90 (female parents). The hybridization success ranged from 3.3% to 5.22% depending upon the parental genotypes (Table D-1).

Table D-1 : Intergeneric and intervarietal hybridization and *in vitro* hybrid embryo germination

Crosses (♀x♂)	Number of pollinated buds	Hybridization success (%)	*In vitro* complet plantlet recovery (%)
T-15-15 x Bandapalera	250	5.2	85
T-15-15 x NP (WR) 15	245	4.08	82
GAUT-82-90 x Bandapalera	240	3.30	90
GAUT-82.90 x NP (WR) 15	250	4.8	87
T-15-15 x *A. lineata*	120	NIL	-
GAUT-82-90 *A. lineata*	80	NIL	-

It was maximum in cross T-15-15 (female parent) and Bandpalera (male parent) and minimum in cross between CAUT-82-90 (female parent) and Bandapalera (male parent).

d Plantlet regeneration from immature embryos

d-1 Effect of salt formulations and growth regulators

Embryos of T-15-15 cultivar excised 16-20 days after anthesis were cultured on different media compositions as listed in Table D-2. On all the media the radicle began to elongate within 5 days followed by the unfolding of the small cotyledons. The appearance of primary leaves was followed by elongation of the epicotyl on all the media. However, variations were observed in the pattern and the rate of regeneration. The complete recovery of plantlet accompanied by small amount of callus at the base was highest (80%) on the medium A. The plantlets regenerated on media E and F (Bladeys formation) were weak and slow growing. However, the plantlets regenerated on MMS formulation with IAA, Kn and CM were healthy and fast growing.

Table D-2 : Plant recovery from cultured embryo of C. cajan (var. T-15-15 16-20 days old embryo)

	Medium	Only callus (%)	Shoot with callus (%)	Complete plantlet formation (%)
A	MMS + 1 mg/l IAA + 0.2 mg/l Kn + 10 % CW + 2 % sucrose	-	20.00	80.00
B	MMS + 1 mg/l 2,4-D + 10 CW + 2 % sucrose	80.00	-	20.00
C	MMS + 0.5 mg/l NAA + 10 % CW + 2 % sucrose	50.00	-	50.00
D	MMS + 10 mg/l NAA + 2 % sucrose	75.00	-	25.00
E	BI basal + 8 % sucrose	-	-	40.00
F	BI basal + 2 % sucrose	-	-	40.00

d-2 Genotypic variation in embryo germination and plantlet regeneration

Embryo germination and plantlet regeneration from eight genotypes (Table D-3) were tested on salts of MS medium with vitamin of B_5 medium supplemented with IAA 1 mg/l, Kn 0.2 mg/l, coconut milk 100 ml/l and sucrose 3.0%. Genotypic variation in plantlet regeneration was considerable (Table D-3). The highest frequency of plantlet recovery (100%) was recorded in T-15-15 and the lowest frequency (20%) was obtained in GAUT-82-99 as well as in ICP 9175. The frequency of embryo germination also varied significantly among genotypes. Embryo germination was highest in BDN_2 (100%) followed by T-15-15 (90%) genotype. However, the germination of T-15-15 embryos was accompanied with slight callusing, whereas no callusing was observed in case of embryo germination in BDN_2. In general, profuse callusing adversely affected embryo germination. For example, maximum callusing (66%) was recorded in GAUT-82-58 which resulted in very low (33%) embryo germination. Similarly, recovery of plantlets was also low in genotypes GAUT-82-99,

Table D-3 : Plant recovery from cultured embryos of eight C. cajan genotypes

Genotype	Callusing (%)	Germinated embryo (%)	Shoots (%)	Rooted plantlets (%)	Shoot length (cm)	No. of root/ shoot
T-15-15	10	90	20	70	2 - 4	3 - 5
GAUT-82-90	40	60	20	40	1 - 5	1 - 2
GAUT-82-99	60	40	20	20	1 - 1	1.0
BDN2	-	100	44	56	1 - 5	2 - 3
AGS-522	50	50	25	25	2 - 3.5	2 - 3
ICP-9175	20	80	60	20	0.5- 1.5	2.0
GAUT-85-44	15	85	34	51	1 - 5	1 - 3
GAUT-82-58	67	33	-	33	0.5- 2.0	1 - 2

AGS-522 and GAUT-82-58 where callus formation was profuse from the embryos. The plantlets of T-15-15 were fast growing attaining maximum shoot length as well as root/shoot as compared to plantlets of other genotypes (Table D-3) (Fig. 17 A & B).

No striking variation was observed in the germination of hybrid embryos (Table D-1) (Fig. 17C). The hybrid plantlets were transferred to pots after 20 days (Fig. 18,A,B,C) and then to the field for further development of F_1 plants.

d-3 In vitro induction of germination in ovules

In order to obtain ovules of uniform age, the flower buds are tagged to note the day of anthesis. The pods of var. T-15-15 were collected from four to twenty days after anthesis and ovules were excised and inoculated on different media (Table D-4) for further development (Fig. 19).

Many of the medium compositions with different combinations of hormones were found to be unsatisfactory for successful development and germination of ovules. In many cases, the

Fig. 17 Plant regeneration from cultured embryo of eight *C. cajan* genotypes (16-20 days old after anthesis)

Age of culture : 2 weeks

Medium : MMS + 1 mg/l IAA + 0.2 mg/l Kn + 100 ml/l CM

From right to left:

A. BDN2, GAUT 82-99, GAUT 822-90, T-15-15

B. GAUT 82-58, GAUT 85-44, ICP-9175, AGS-522

X1.2

C. Plant regeneration from hybrid embryo of *C. cajan* (16 days old after pollination)

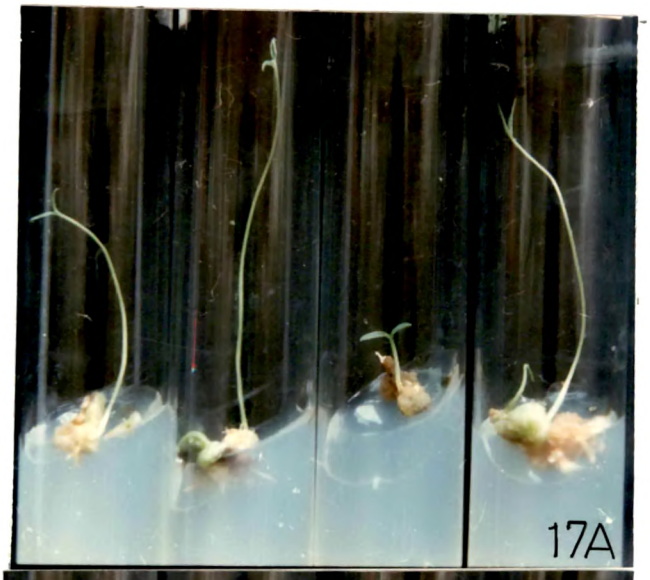
Age of culture ; 2 weeks

Medium : MMS + 1 mg/l IAA + 0.2 mg/l Kn + 100 ml/l CM

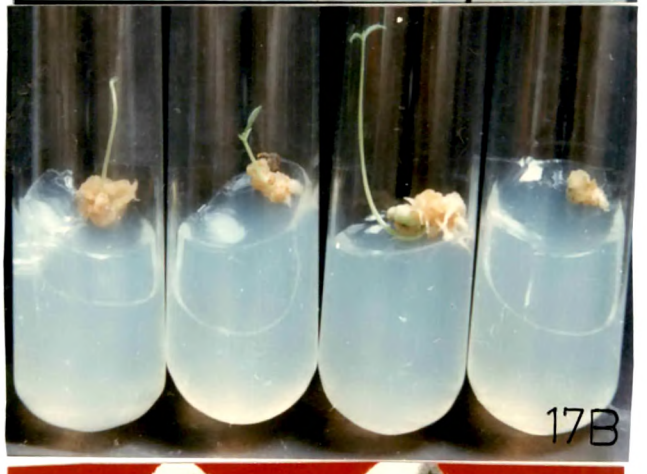
From right to left :

T-15-15 x NP (WR)15, T-15-15 x Bandapalera, GAUT-82-90 x Bandapalera, GAUT-82-90 x NP (WR)15

X1.4



17A



17B

17C

Fig. 18 The F_1 plant of C. cajan (20 days after incubation)

A (1) GAUT-82-90 x NP (WR) 15

(2) GAUT-82-90 x Bandapalera

B (3) T-15-15 x Bandapalera

(4) T-15-15 x NP (WR) 15

C The F_1 plant (Three month old)

Left : GAUT-82-90 x NP (WR) 15

Right : T-15-15 x NP (WR) 15

18A

18B

18C

planted ovules initially swelled slightly, then shrivelled, became hard, turned brown or black, and eventually died. Although, in some cases though the ovules remained viable and green for about 20-25 days, ovules did not develop and germinate into plantlets. Ovules of 8 days or older when planted on media supplemented with auxin, kinetin, casin hydrolysate and coconut milk individually or in combinations formed white brown callus from placenta in 20% to 40% of the cases. On most of the media, the ovules failed to germinate. However, 20-25% ovules germinated and developed into either single shoot or multiple shoots after one and half months of incubation on Blady's basal media with 2 or 8% sucrose or supplemented with kinetin, GA_3, casien hydrolysate or on salts of MS medium and vitamins of B_5 medium supplemented with IAA or NAA, kinetin and casien hydrolate.

It was observed that successful development and germination of ovules depended on the age of ovules besides the medium composition. No germination was recorded when ovules were less than 6 days or more than 12 days old. These

Table D-4 : *In vitro* germination response of ovules after different days of anthesis (var. T-15-15)

Media combination	Ovule age after anthesis					
	4 days	6 days	8 days	12 days	16 days	20 days
1	2	3	4	5	6	7
1. Blady's + 2 % sucrose	Slight swelling brown, dry	Slight, Green germination (20%)	Slight swelling brown, germination (20%)	Brown or black shrivelet, hard	Swelling, brown black, shriveled	Brown, black
2. Blady's + 8 % sucrose	Swelling, brown, dry	Brown, shriveled, hard, germination (20%)	Brown, black shriveled, hard	-do-	-do-	-do-
3. Bl + 0.1 mg/l Kn + 0.01 mg/l GA_3 + 100 mg/l CH + 2 % sucrose	Brown, black	Brown, shriveled, hard	-do-	-do-	-do-	-do-
4. Bl + 0.1 mg/l Kn + 0.05 mg/l GA_3 + 100 mg/l CH + 2 % sucrose	-do-	-do-	-do-	-do-	-do-	-do-
5. Bl + 0.1 mg/l Kn + 0.1 mg/l GA_3 + 100 mg/l CH + 2 % sucrose	-do-	-do-	-do-	-do-	-do-	-do-
6. Bl + 0.5 mg/l Kn + 0.01 mg/l GA_3 + 100 mg/l CH + 2% sucrose	-do-	-do-	-do-	Brown, black	Brown, black shriveled, brown callus	-do-
7. Bl + 0.5 mg/l Kn + 0.05 mg/l GA_3 + 100 mg/l CH + 2% sucrose	Brown, black, dry, no response	Shriveled, brown, dry, no response	Brown, black, slight brown callus, germination (20 %)	Brown, black, slight brown callus germination (20 %)	Black, hard dry, no response, callus	Black, dry no response
8. Bl + 0.05 mg/l Kn + 0.1 mg/l GA_3 + 100 mg/l CH + 2% sucrose	-do-	-do-	Brown, black, shriveled, hard brown callus	Brown, black brown callus	-do-	-do-
9. Bl + 1.0 mg/l Kn + 0.01 mg/l GA_3 + 100 mg/l CH + 2 % sucrose	-do-	-do-	-do-	-do-	-do-	-do-
10. Bl + 1.0 mg/l Kn + 0.05 mg/l GA_3 + 100 mg/l CH + 2 % sucrose	-do-	-do-	-do-	-do-	-do-	-do-

(Contd.)

(Contd.)

1	2	3	4	5	6	7
11. BI + 1.0 mg/l Kn + 0.1 mg/l GA_3 100 mg/l CH + 2% sucrose	Brown, black, dry no response	Shriveled, brown, dry, no response	Brown, black, shriveled, hard, brown callus	Brown. black brown callus	Black hard, dry no response callus	Black, dry, no response
12. MMS + 10 mg/l NAA + 2%	-do-	-do-	-do-	Brown, black, shriveled, brown	Brown. black shriveled brown	Brown, black brown callus
13. MMS + 0.2 mg/l Kn + 0.05 mg/l NAA + 500 mg/l CH+0.4 % charcoal + 2 % sucrose	-do-	No response	No response	Shriveled brown, germination (20%), callus, multiple shoot	Brown, black shriveled, brown callus	-do-
14. MMS + 0.02 mg/l IBA + 0.1 mg/l GA_3 7 % CW + 2 % sucrose + 0.4% charcoal	-do-	-do-	-do-	Brown, black	Brown, black shriveled brown callus	-do-
15. MMS + 1 mg/l IAA + 0.5 mg/l Kn + 100 mg/l CH + 2 % sucross + 0.4 % charcoal	-do-	Brown. shriveled callus, germination (20%)	Brown, shriveled, callus, germination multiple shoot (25%)	-do-	-do-	-do-
16. MMS + 7% CW + 2% sucrose + 0.4 % charcoal	-do-	Brown, black, dry	Brown, black dry	-do-	Brown, black. shriveled no cellus	Brown, black, no callus
17. MMS + 200 mg/l AdS + 2 % sucrose + 0.4 % charcoal	-do-	-do-	Brown, black germination (25 %)	Brown, black dry	-do-	-do-

ovules germinated and developed into either single shoot or multiple shoots after one and half months of incubation (Fig. 20). Maximum response (25%) germination with multiple shoots was recorded with 12 days old ovules on MMS medium supplemented with 0.2 mg/l Kn, 0.05 mg/l NAA, 500 mg/l CH, 0.4% charcoal and 2.0% sucrose.

Germinated ovules with single or multiple shoots were transferred for rooting and further growth of shoots on ½ MMS basal medium supplemented with 0.1 mg/l IBA, 0.05 mg/l GA_3 and 2% sucrose. Root primordia were observed within a week of inoculation.

(E) PATHOLOGICAL STUDIES

e-1 Dose determination of the fungal mycelium and its culture filtrate at whole plant level

The influence of inoculum size of F. udum. on Cajanus cajan var. T-15-15 was studied by varying the concentration of the fungus mycelium and culture filtrate using the liquid culture method. The mycelium concentration from 0.1 to 30% (w/v) and CF concentration from 0.1% to 80% (v/v) were used for the purpose. About one month old seedlings were inoculated to each

Fig. 19 The pods and ovules of var. T-15-15 on different days after anthesis (0, 2, 4, 6, 8, 12, 16, 20, 25, 30 days)

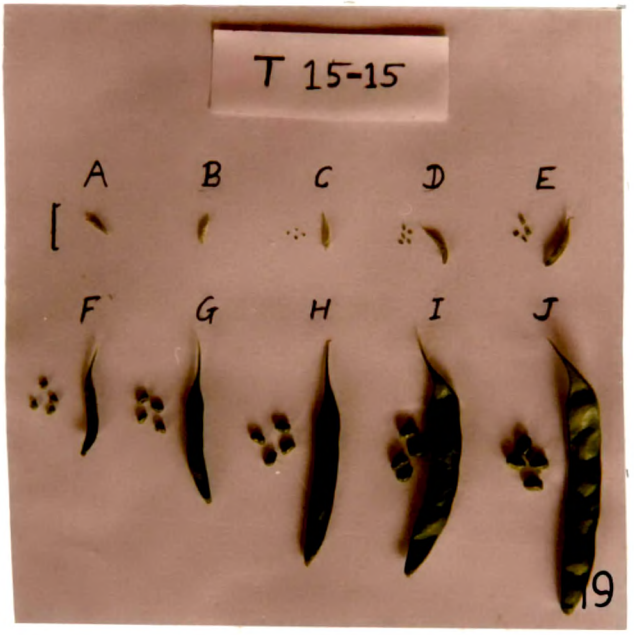
X1.4

Fir. 20 Germinated ovule (6-8 days after anthesis) of var. T-15-15

Age of culture : 6 weeks

Medium : MMS + 0.2 mg/l Kn + 0.05 mg/l NAA + 500 mg/l CH + 0.4 % charcoal + 2 % sucrose

X1.5


T 15-15
A
B
C
D
E
F
G
H
I
J
19


20

concentration. The observations of the following characters were recorded during incubation period: (i) yellowing of the lower leaves, (ii) vein clearing in upper leaves, (iii) drying up of leaves and (iv) wilting of the entire plant. Vein clearing, yellowing of lower leaves and wilting of plant were recorded at regular intervals between 12 to 72 h after inoculation.

All the three parameters showed visible differences between 12 to 18 h after inoculation in higher levels of CF. Appearance of the disease symptoms was noted much earlier in CF treatments than with direct fungal mycelium infection. Wilting of leaves as well as entire plants were observed cent per cent with 40% or above levels of CF, while more than 95% effect was recorded at 25% or above levels of fungus mycelium The correlation of variability for wilting for leaves and entire plants was satisfied with CF but not with fungus mycelium. Based on this results, 15% (v/v) level of CF and 5% (v/w) level of mycelium were fixed as the optimal level for the pathogenecity test (Table E-1) (Fig. 21).

Table E-1 : Effect of different concentrations of CF (v/v) and fungal mycelium (w/v) on wilt induction in *C. cajan* (var. T-15-15)

Dose (%), (v/v or w/v)	Wilt index* in CF (%)	Wilt index* in mycelium (%)
Control	0.0	0.0
0.1	1.0	10.3
0.5	2.5	5.5
1.0	10.25	20.5
5.0	21.5	58.25
10.0	21.5	58.6
15.0	58.25	58.6
20.0	64.0	78.2
25.0	85.5	95.0
30.0	97.5	99.0
40.0	100.0	-
60.0	100.0	-
80.0	100.0	-

* Observations were noted between 12 to 48 h.

Fig. 21 Wilting of *C. cajan* (var. T-15-15) plants treated with :

a. 15 % v/v culture filtrate of *F. udum*

b. 5 % w/v fungal mycelia

c. Control

X1.2


a
b
c
21

e-2 Screening of pigeonpea varieties for disease susceptibility to the pathogen and its culture filtrate

C. cajan vars. T-15-15, BDN-2, AGS-498, Bandapalera, ICP-7336, GAUT-82-90, GAUT-82-99, NP (WR) 15 and G-78-3 were subjected to 15% (v/v) level of culture filtrate or 5% (w/v) level of fungus mycelium to determine differences in the susceptibility of varieties to the pathogen and its CF. Five plants for control and 6 plants for each of the treatments were used for the purpose (Table E-2).

Symptoms were recorded at regular intervals between 12 to 72 h after inoculation. The symptoms such as vein clearing and yellowing of lower leaves were visible after 12 h of inoculation in susceptible varieties. On the other hand, more tolerant varieties, such as ICP 7336, NP (WR) 15, BDN-2 and Bandapalera exhibited the symptoms only after 48 h of inoculation. The plants took 2 days for complete wilting when inoculated with culture filtrate or fungal mycelium. Control plants of all varieties remained healthy throughout the

Table E-2 : *In vivo* varietal screening with CF and mycelium of *F. udum*

Genotypes	Wilt index (%)	
	With CF (15%)	: With mycelium (5 %)
T-15-15	81.5 (± 8.8)	84.2 (±10.2)
BDN2	27.0 (± 8.0)	32.0 (± 8.1)
AGS-498	56.9 (± 7.4)	64.0 (± 9.8)
Bandapalera	30.25(±11.6)	42.2 (±10.8)
ICP-7336	13.8 (± 3.9)	30.5 (± 7.1)
CAUT-82-90	81.25(±16.9)	81.5 (± 8.1)
CAUT-82-99	58.25(± 9.5)	82.9 (±13.1)
NP (WR) 15	20.0 (± 8.5)	26.5 (±16.2)
G-78-3	66.5 (±13.1)	73.8 (±14.5)

* The observations were recorded after 12 to 48 h of inoculation.

experiment and showed on evidence of chlorophyl degradation.

The varieties were ranked based on their relative tolerance to the pathogen and its culture filtrate. The ranking order of the nine varieties was calculated on the basis of their wilt index (sec Materials and Methods, e-22) and was as under:

Varietal ranking with culture filtrate

a. Susceptible : T-15-15 > GAUT-82-90 > G-78-3 > GAUT-82-99 > AGS-498

b. Tolerant : Bandapalera < BDN-2 < NP(WR)15 < ICP 7336

Varietal ranking with fungus mycelium

a. Susceptible : T-15-15 > GAUT-82-90 > GAUT-82-90 > G-78-3 > AGS-498

b. Tolerant : Bandapalera < BDN-2 < ICP-7336 < NP (WR) 15.

The ranks of varieties obtained by two different methods were subjected to coefficient of correlation analysis (Redei 1982). The spearman rank correlation for the ranking of varieties between direct infection with the pathogen and with the culture filterate was significant and positive (Fig. 22).

Fig. 22 Correlation of ranking of varieties between direct infection with pathogen and its culture filtrate

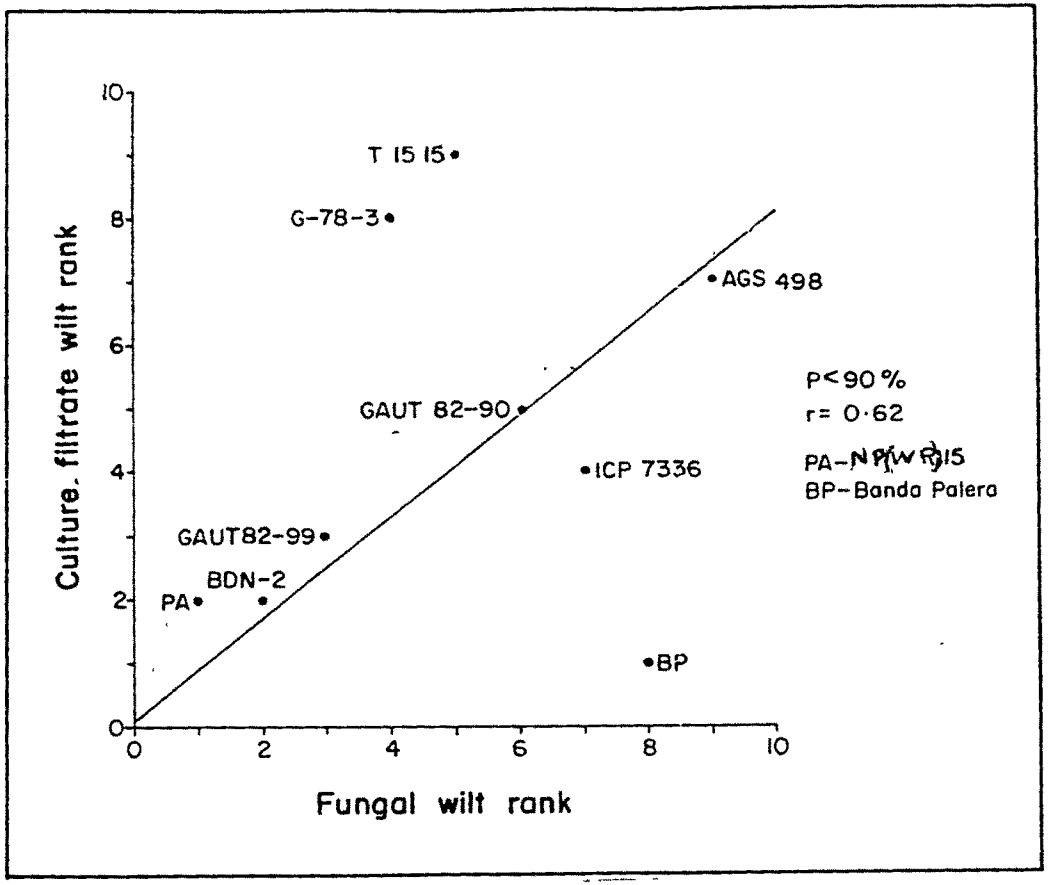

Culture. filtrate wilt rank
Fungal wilt rank
T 15 15
G-78-3
AGS 498
GAUT 82-90
ICP 7336
GAUT82-99
BDN-2
PA
BP
P<90%
r= 0·62
PA-NP(WR)15
BP-Banda Palera
0
2
4
6
8
10

Fig. 22

e-3 Varietal screening by leaf disc assay

Leaf discs (8 mm diameter) obtained from surface sterilized young leaves of suceptible variety T-15-15 were inoculated in various concentrations of culture filtrate for the determination of LD 50. The inhibiting effect of the CF was expressed in terms of chlorophyll degradation in cultured leaf discs. Chlorophyll estimation was carried out every 24 h till 72 h. The optimum time period for exposure of leaf discs to culture filtrate was found to be 48 h. LD 50 was recorded at 5% (v/v) CF. This data was used to screen eight varieties of C. cajan and two species of Atylosia for the effect of CF on chlorophyll degradation in leaves. On the basis of loss of chlorophyll from the leaf discs the varieties are rated susceptible to tolerant : ICPL 8010> T-15-15≥ Pusa ageti > A. cajanifolia >ICP-87 > GAUT-82-90 >GAUT-88-7 > BDN-2 > A. lineata >GAU-88-10 (Table E-3).

e-4 Dose determination of culture filtrate for cotyledon explants grown in vitro

Distal halves of sterilized cotyledons of the reportedly susceptible var. T-15-15 were

Table E-3 : Varietal screening by leaf disc assay method with CF (5 % v/v) of *F. udum*

Genotypes	Chlorophyll content (mg/gm Fr. wts)		
	Control	CF	Loss of chlorophyll
T-15-15	4.37	3.16	1.21
BDN-2	3.59	3.43	0.16
GAUT-88-10	3.90	3.80	0.10
GAUT-88-7	2.90	2.70	0.20
GAUT-82-90	2.98	2.70	0.28
ICPL-84010	4.52	3.06	1.46
ICPL-87	3.95	3.64	0.31
Pusa Ageti	3.30	2.62	0.68
Atylosia cajanifolia	3.04	2.72	0.32
A. lineata	3.02	2.88	0.14

inoculated in various concentrations of CF on MmS medium (full) and mmS (½) with MR (modified Richard's) induction medium for the determination of LD-50. The inhibitory effect of the CF was expressed in terms of per cent responsive cotyledons, degradation of colour pigments from explant, degree of embryo induction frequency in cotyledons and fresh and dry weights of inoculated cotyledons. These observations were noted 20-25 days after inoculation.

The CF at concentration of 10% had very little inhibitory effect on growth of cotyledons, degradation of colour pigments and embryo induction frequency in cotyledons. Above 10% level of CF showed pronounced inhibitory effect on responsive cotyledon, embryo induction frequency and growth of cotyledons. Necrosis, inhibition of embryo induction and growth were recorded in cotyledons incubated in 40% or above CF levels on both the media tested (Table E-4). The chlorotic symptoms were observed in cultures on all the media containing ½ MMS and MR induction medium with CF at different levels (Table E-5). Therefore, twenty per cent (v/v) level of CF with MMS medium was considered as the optimal medium for pathogenecity test.

Table E-4: Dose determination of CF of F. udum on MMS medium for var. T-15-15

Conc. of CF in medium (%)	Responsive cotyledons (%)	Colour development in cotyledons (%)				Frequency of embryo induction in cotyledons (%)				Av. Fresh wts/Coty/ (mg)	Av. Dry wts/Coty. (mg)
		A	B	C	D	0	+	++	+++		
0 Control	80.00 (±14.1)	40.00	33.33	6.66	20.00	13.33	20.00	26.66	20.00	369.8 (±171.5)	47.7 (±11.5)
5	80.00 (±16.3)	33.33	33.33	13.33	20.00	13.33	33.33	20.00	13.33	338.3 (±140.9)	43.6 (±10.5)
10	66.66 (± 9.4)	26.66	26.66	13.33	33.33	33.33	26.00	6.66	0.00	207.6 (± 68.7)	30.2 (± 5.2)
20	46.66 (± 9.4)	0.00	20.00	33.33	46.33	40.00	6.66	0.00	0.00	160.9 (± 71.6)	28.3 (±14.6)
30	26.66 (± 9.4)	0.00	6.66	13.33	80.00	26.66	0.00	0.00	0.00	131.2 (± 67.6)	27.2 (± 6.8)
40	20.00 (±16.3)	0.00	0.00	20.00	80.00	20.00	0.00	0.00	0.00	80.3 (± 24.2)	25.6 (± 3.3)
60	0.00 (0.0)	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	86.3 (± 21.2)	23.6 (± 5.8)
80	0.00 (0.0)	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	80.8 (± 15.6)	23.5 (± 3.9)

Value

A = Dark green
B = Pale green
C = Whitish brown
D = Brown

0 = Nil frequency
+ = Low frequency
++ = Moderate frequency
+++ = High frequency

Value in parenthesis indicates ± SD.

Table E-5 : Dose determination of CF F. udum on ½ MMS + MR medium for var. T-15-15

Conc. of CF in medium (%)	Responsive cotyledons (%)	Colour development in cotyledons (%)				Frequency of embryo induction in cotyledons (%)				Av. fresh wts/coty. (mg)	Av. dty wts/coty. (mg)
		A	B	C	D	0	+	++	+++		
0 (Control)	77.77 (±15.2)	27.77	16.66	33.55	22.22	16.66	22.22	22.22	16.66	302.0 (±179.2)	38.1 (±22.6)
5	72.68 (± 8.2)	15.78	15.78	36.84	26.31	21.05	26.31	15.78	10.52	291.2 (±127.6)	36.0 (±15.8)
10	63.15 (±10.8)	5.26	10.52	47.36	36.84	31.57	21.05	10.52	0.00	198.5 (± 97.5)	28.7 (±14.1)
20	44.44 (± 5.0)	0.00	11.11	33.33	55.55	33.33	11.11	0.00	0.00	155.0 (± 49.8)	27.1 (± 8.7)
30	25.00 (± 8.6)	0.00	0.00	25.00	75.00	20.00	5.00	0.00	0.00	122.4 (± 37.4)	26.0 (± 6.4)
40	15.78 (± 9.6)	0.00	0.00	15.78	84.21	15.78	0.00	0.00	0.00	80.01 (± 29.4)	25.2 (± 9.3)
60	0.00 (± 0.0)	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	85.0 (± 20.7)	23.6 (± 5.7)
80	0.00 (± 9.0)	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	84.4 (± 15.6)	23.5 (± 3.9)

Value

A = Dark green
B = Pale green
C = Whitish brown
D = Brown

0 = Nil frequency
+ = Low frequency
++ = Moderate frequency
+++ = High frequency

Value in parenthesis indicates ± SD.

In all cases, the cultures grown in control (without CF) conditions exhibited healthy cotyledons and recorded maximum responsive cotyledons and higher frequency of embryo induction in cotyledon.

e-5 Use of CF of *F. udum* as selection pressure in *in vitro* culture of cotyledons of *C. cajan* varieties

The protocol for high degree of plant regeneration via somatic embryogenesis was developed as described in earlier experiments in this chapter through three stages of explant subculturing. The CF (20% v/v) was used as selection pressure for *in vitro* culture with 0.8% agar based MMS culture medium. The CF filter sterilized and mixed with previously autoclaved culture medium. No CF was added to the control medium. The pH of all the media was adjusted to 5.8.

The distal halves of cotyledons of five varieties of pigeonpea were cultured on CF added MMS media. The data on per cent responsive cotyledons, degradation of colour pigments in explants, degree of embryo induction frequency in per cent cotyledons and fresh and dry weights

of cotyledons for all the varieties tested at three different stages of embryo development are presented in Table E-6,7,8.

The effect of CF on per cent responsive cotyledons at each stages of embryo development was recorded 20 days after inoculation on culture medium. At first and second stage, T-15-15 and CAUT-82-90 showed 50% or less responsive cotyledons. On the other hand, other varieties registered more than 67% responsive cotyledons. The inhibitory effect of CF was more pronounced at third stage where only two varieties viz., NP (WR) 15 and BDN-2, recorded more than 50% responsive cotyledons. At this stage, T-15-15 registered minimum (20%) and NP (WR) 15 showed maximum (72.6%) responsive cotyledons. Such marked differences in cotyledon response were not found amongst varieties on the control medium.

The effect of CF on the degradation of colour pigments was considerable at all the stages of cotyledon culture in all five varieties. It was higher in T-15-15 and CAUT-82-90 as compared to BDN2, Bandapalera or NP (WR) 15.

The lowest degradation of pigements was observed in var. NP (WR) 15. At the final maturation stage, the highest (80%) brown and dead cotyledons were observed in var. T-15-15 and the lowest (26%) in var. NP (WR) 15.

The extend of embryo induction in per cent cotyledons was adversely affected by CF in all the varieties. In var. T-15-15 and GAuT-82-90, this adverse effect was more as compared to NP (WR) 15, Bandapalera or BDN2 varieties, with NP (WR) 15 registering the highest degree of frequency of embryo induction.

The results showed an increase in fresh and dry weights of cotyledons of all the five varieties inoculated on control medium at all the stages. The cultured cotyledons were found to be highely sensitive to the presence of CF in culture medium, with regard to the frersh and dry weights. All the five varieties showed considerable reduction in fresh and dry weights of cotyledons due to CF in culture medium at the first two stages. At the final meaturation stage, more than 50% reduction in fresh and dry weights was noticed in var. T-15-15 and

Table E-6 : Effect of CF of *F. udum* on somatic embryo induction in the cotyledonary explants of different genotypes of *C. cajan*

Genotypes		Responsive cotyledons (%)	Colour development in cotyledons (%)				Frequency of embryo induction/ cotypedons (%)				Av. Fresh wts/cotyledon (mg)	Av. Dry wts/cotyledon(mg)
			A	B	C	D	0	+	++	+++		
T-15-15	C	80.00 (±16.3)	53.33	26.66	6.66	13.33	0.00	13.33	53.33	13.33	361.2 (±65.6)	40.4 (±5.8)
	T	47.83 (±12.5)	13.04	8.69	26.08	52.17	30.43	8.69	8.69	0.00	116.8 (±22.5)	30.5 (±4.7)
GAUT-82-90	C	80.00 (±14.1)	55.00	20.00	5.00	20.00	5.00	10.00	45.00	20.00	249.2 (±79.9)	30.6 (±.4.7)
	T	48.15 (±10.6)	11.11	11.11	25.29	51.85	22.22	14.81	11.11	0.00	96.66 (±18.4)	26.16 (±4.8)
Bandapalera	C	89.47 (±11.4)	63.15	21.05	5.26	10.52	0.00	10.53	26.31	52.63	301.09 (±65.1)	38.75 (±6.7)
	T	73.91 (± 5.6)	43.48	17.39	13.04	26.08	13.04	26.08	21.74	13.04	152.43 (±53.8)	34.28 (±3.9)
NP (WR) 15	C	95.0 (± 8.6)	75.00	20.00	0.00	5.00	0.00	10.00	25.00	60.00	226.4 (±42.8)	24.4 (±5.4)
	T	87.50 (±12.5)	35.00	40.00	20.00	12.50	20.00	17.50	27.50	25.00	120.2 (±38.2)	23.5 (±5.8)
BDN2	C	85.71 (± 8.3)	57.14	23.14	4.76	9.50	4.76	14.28	23.81	42.85	150.25 (±60.9)	22.75 (±5.6)
	T	67.65 (± 9.5)	35.29	20.58	20.58	32.25	29.41	20.58	8.82	8.82	98.7 (±22.1)	20.5 (± 4.8)

Value

A = Dark green
B = Pale green
C = Whitish brown
D = Brown

0 = Nil frequency
+ = Low frequency
++ = Moderate frequency
+++ = High frequency

C = Control
T = Treated with 20% sterilized CF of *F. udum*

Induction medium

MMS + 5 mg/l BAP + 0.5 mg/l Kn + 50 mg/l AdS

Value in parenthesis indicates ± SD.

Table E-7 : Effect of CF on somatic embryo maturation from the cotyledonary explants of different genotypes of C.cajan

Genotypes		Responsive cotyledons (%)	Colour development in cotyledon (%)				Frequency of embryo induction in cotyledons (%)				Av. fresh wts/ coty. (mg)	Av. dry wts/ coty. (mg)
			A	B	C	D	0	+	++	+++		
T-15-15	C	85.71 (±9.4)	57.14	28.57	0.00	14.28	14.28	14.28	28.56	28.57	608.7 (±48.5)	55.6 (±4.4)
	T	57.14 (±8.3)	0.00	28.50	28.50	42.85	14.28	28.57	14.28	0.00	158.6 (±83.05)	36.3 (±10.5)
CAU-82-90	C	86.66 (±9.4)	60.00	26.66	6.66	6.66	6.66	6.66	40.00	33.33	630.2 (±41.6)	53.2 (±4.0)
	T	44.44 (±15.7)	11.11	11.11	22.22	55.55	33.33	11.11	0.00	0.00	170.8 (±44.2)	23.8 (±4.9)
Bandapalera	C	91.66 (±8.0)	66.66	25.00	8.33	0.00	8.33	8.33	33.33	41.66	675.5 (±71.3)	61.4 (±6.63)
	T	83.33 (±11.7)	50.00	16.66	16.66	16.66	8.33	16.66	33.33	25.00	308.6 (±57.3)	48.2 (±13.0)
NP (WR) 15	C	96.10 (±8.0)	76.00	16.00	8.00	0.00	0.00	8.00	24.00	64.00	472.4 (±39.9)	48.8 (±4.1)
	T	94.44 (±8.6)	66.66	16.66	11.11	5.55	11.11	11.11	27.77	44.44	255.3 (±38.5)	26.8 (±6.5)
BDN2	C	92.30 (±9.2)	69.23	23.00	7.66	0.00	0.00	15.38	23.07	53.84	304.5 (±27.7)	45.4 (±4.4)
	T	73.33 (±9.4)	33.33	20.00	20.00	26.66	26.66	26.66	13.33	6.66	141.5 (±43.6)	22.7 (4.6)

Maturation medium : MMS + 0.5 mg/l BAP + 0.05 mg/l Kn + 5.0 mg/l AdS

Explainations as given in Table E-6

Table E-8 : Effect of CF on somatic embryo germination from cotyledonary explants of different genotypes of C. cajan

Genotypes		Responsive cotyledons (%)	Colour development in cotyledon (%)				Embryos frequency in cotyledons (%)				Av. Fresh wts/ coty (mg)	Av. dry wts/ coty. (mg)
			A	B	C	D	0	+	++	+++		
T-15-15	C	66.6 (±10.8)	25.00	33.33	8.3	33.3	0.0	8.3	16.6	41.6	786.7 (±561.9)	80.7 (±51.1)
	T	20.0 (± 0.0)	0.00	0.00	20.0	80.0	0.0	20.0	0.0	0.0	281.5 (±17.5)	24.0 (1.3)
CAUT-82-90	C	63.6 (±9.4)	27.3	27.3	9.1	36.4	0.0	0.0	18.2	45.2	690.7 (±640.5)	68.7 (±57.9)
	T	25.0 (±20.0)	0.0	0.0	25.0	75.0	0.0	25.0	0.0	0.0	215.3 (±27.4)	23.6 (±2.0)
Bandapalera	C	75.0 (±15.0)	41.5	25.0	8.3	25.0	0.0	8.3	16.6	50.0	878.0 (±265.4)	84.5 (±14.2)
	T	50.0 (±8.1)	0.0	20.0	30.0	50.0	10.0	20.0	20.0	0.0	639.7 (±354.0)	65.5 (±34.5)
NP (WR) 15	C	78.6 (±10.8)	42.8	28.5	7.1	21.4	0.0	7.1	21.4	50.0	605.0 (±162.6)	69.5 (±12.6)
	T	72.6 (± 8.8)	41.76	7.6	23.7	26.0	0.0	23.1	7.7	41.8	514.5 (±280.1)	52.7 (±26.0)
BDN2	C	75.0 (±15.0)	41.6	25.0	8.3	25.0	0.0	8.3	25.0	41.6	348.0 (±71.9)	49.8 (± 8.1)
	T	66.6 (±14.2)	25.0	8.3	33.3	33.3	0.0	25.0	16.6	25.0	183.5 (±29.8)	27.5 (± 2.0)

Germination medium : MMS + 0.1 mg/l IBA + 0.05 mg/l GA_3

Explanations as given in Table E-6

GAU-822-90. The suppression in fresh and dry weights was less than 50% in all the other varieties with NP (WR) 15 registering the lowest reduction in growth (14.96% in fresh weight and 11.43% in dry weight).

From the data obtained on per centresponsive cotyledons and frequency of embryo induction in cotyledons, var. NP (WR) 15 found found to be having high regeneration capability as compared to other varieties. These results indicated NP (WR) 15, BDN22 and Bandapalera varieties as disease resistant and T-15-15 and GAUT-82-90 as disease susceptible.

e-6 Screening of F_2 generation and its parents for wilt disease tolerance

F_1 plants were raised through embryo rescue and planted in pots. To collect selfed seeds (F_2 seeds), young inflorescences were bagged before anthesis to prevent cross pollination. Ten plants of F_2 and each of its parents were subjected to 15% (v/v) level of CF to determine relative tolerance to wilt disease at one month old seedling stage. Symptoms were recorded at regular intervals between 12 to 48 h after inoculation.

The F_2 generation and its parents were ranked on the basis of their relative tolerance to CF. Using wilt index the ranking order of F_2 and its parents was as under:

F_2 : NP (WR) 15 x GAUT-82-90 > NP (WR) 15 x T-15-15 > bandapalera X GAUT-82-90 > Bandapalera X T-15-15

Parents : NP (WR) 15 > Bandapalera > GAUT-82-90 > T-15-15.

e-6 Anatomical study of disease infested pigeeonpea plant

The hand sections of disease infected stemm and root were stained with toludine blue and lactophenol cotton blue to confirm the presence of fungal mycelia in the xylem vessels. No blockage of vessels by the fungal mycellia was observed in the hand section of stem. On the other hand, roots showed blockage of xylem vessels. No such anatomical changes were observed in the control plants (Fig. 23).

Fig. 23 Transverse section of the stem of C. cajan (var. T-15-15)

a. Vessel elements of control plants

X200

b. Vessel elements of the plant treated with culture filtrate (15%, v/v) of F. udum

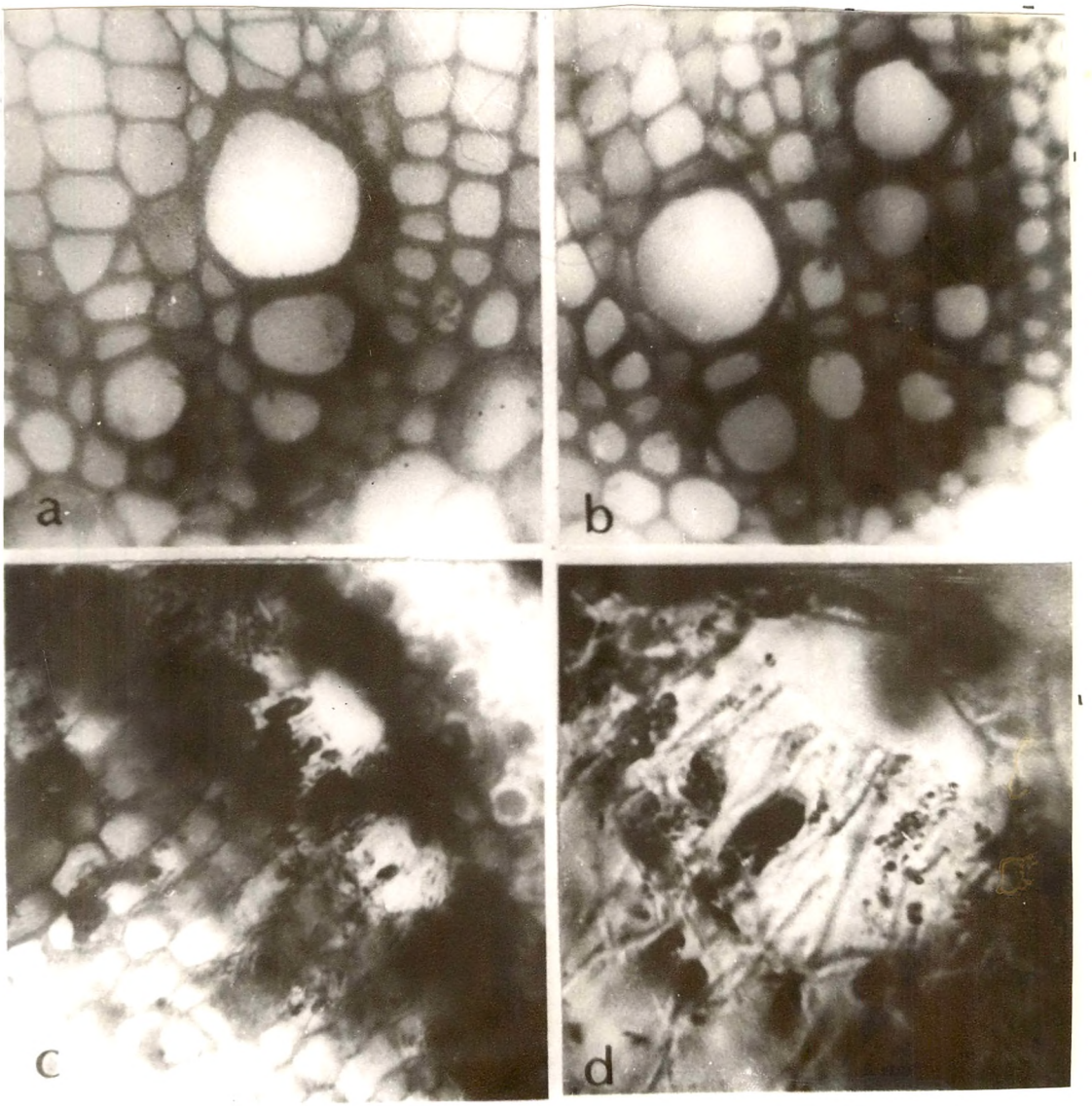
X200

c. Vessel elements of the plant treated with the pathogen F. udum showing growth of fungal mycelia into the lumen.

X200

d. A magnified vessel element from fig. c showing blockage of the lumen by profuse growth of fungal mycelia

X500


a
b
c
d