

## STUDIES ON CALLUS INITIATION AND PLANTLET REGENERATION IN FIELD BEAN (*LABLAB PURPUREUS* (L.) SWEET)

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### SUMMARY

The studies on callus initiation and plantlet regeneration in field bean were undertaken by using B<sub>5</sub> medium, ten genotypes and different growth regulators. The stem and leaf explants were used for callus induction, while shoot-tip and cotyledonary node explants were used for plantlet regeneration. The response for per cent callus induction and plantlet regeneration varied with genotype, explants and growth regulators. It was observed that the genotype Konkan Wal 1, explant stem and growth regulator combination of 2.5 mg/l IAA + 5.0 mg/l BA recorded maximum callus induction. The genotype Konkan Wal 1, explant shoot-tip and growth regulator level of 0.1 mg/l BA recorded maximum plantlet regeneration.

**Key words:** Callus, field bean, growth regulator, regeneration

### INTRODUCTION

The field bean [*Lablab purpureus* (L.) Sweet] is an important legume grown in India. Callus cultures are used for inducing genetic variability and recovery of variant plants. The plants regenerated from callus show considerable amount of variation known as somaclonal variation. These variations can be directly used in crop improvement [Karp 1995, Jain 2000]. *In vitro* culture of shoot-tip meristems has many applications in crop improvement programs. It can be used to obtain virus free material, clonal propagation and germplasm conservation [Kantha *et al.* 1981, Kale 2004]. Not much work has been carried out on *in vitro* culture of field bean. The present investigation was undertaken to study callus initiation and plantlet regeneration in field bean.

### MATERIALS AND METHODS

The seeds of ten field bean genotypes, *viz.* Konkan Wal 1, Konkan Wal 2, Hebbal 3, Arka Vijay, ACCW

113, ACCW 116, ACCW 147, ACCW 148, ACCW 165 and ACCW 166 were first washed with teepol in running tap water for 10-15 minutes. The seeds were then rinsed in 70% ethanol for 2-3 minutes and washed 4-5 times with distilled water. The surface sterilization was done with 0.1% HgCl<sub>2</sub> for 5 minutes. The seeds were finally washed thoroughly with distilled water for 4 to 5 times. The seeds were then inoculated on B<sub>5</sub> medium without any growth regulators. The stem and leaf explants were taken for callus induction while shoot-tip and cotyledonary node for plantlet regeneration. The explants of about 0.5 cm long were excised from 7-day old seedlings and inoculated on B<sub>5</sub> medium containing 3 per cent sucrose and 0.8 per cent agar supplemented with IAA and BA at various concentrations and combinations. The pH of the medium was adjusted to about 5.8 before autoclaving at 15 psi for 15 minutes. The cultures were kept in darkness for callus induction while they were maintained at 25 ± 2°C in light (1600 lux from cool white fluorescent tubes) for plantlet regeneration. The data were recorded on per cent callus initiation and plantlet regeneration.

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## RESULTS AND DISCUSSION

### Callus Induction

The genotype of the donor plant may be the most important factor in callus induction [Jain and Chopra 1988]. The data showing per cent response of different genotypes of field bean to callus induction using stem and leaf explants on B<sub>5</sub> medium are presented in Table 1. It is observed that, the per cent response of genotypes ranged from 88.06 to 97.39. The maximum response (97.39%) was recorded by Konkan Wal-1, while minimum response (88.06%) was recorded by ACCW-147. The genotypes Konkan Wal-1 and Hebbal-3 were found to be statistically significant over all the remaining genotypes. The genotypes, Konkan Wal-2 and Arka Vijay were at par with each other, while Konkan Wal-2 was significantly superior to the remaining genotypes.

The maximum response (91.87%) for callus induction was recorded by stem explant, while minimum response (91.51%) was recorded by leaf explant. With the stem

explant, maximum response (96.40%) was recorded by Konkan Wal-1, while minimum (85.00%) was recorded by Konkan Wal-2. With the leaf explant, maximum response (98.38%) was also recorded by Konkan Wal-1, while minimum response (84.60%) was recorded by Arka Vijay.

The effect of growth regulator combinations on callus induction of field bean genotypes are presented in Table 2. It is evident that, growth regulator combinations created variable response for callus induction. The maximum response (92.90%) for callusing was recorded in growth regulator combination 2.5 mg/l IAA + 5 mg/l BA while, the minimum response (90.56%) was recorded by growth regulator combination 2.5 mg/l NAA + 5 mg/l BA. The percent callus induction ranged from 85.86 to 99.33, 86.63 to 98.78, 88.25 to 98.04 and 88.19 to 97.08 with growth regulator combinations of 2.5 mg/l IAA + 5 mg/l BA, 2.5 mg/l NAA + 5 mg/l BA, 1.0 mg/l IAA + 10 mg/l BA and 1.0 mg/l NAA + 10 mg/l BA, respectively. The callus developed from stem explant on MS + 2.5 mg/l IAA + 5.0 mg/l BA is shown in fig. 1.

**Table 1.** Genotypic variability in relation to explants for per cent callus induction on B<sub>5</sub> medium.

Genotypes	Stem	Leaf	Mean
Konkan Wal 1	96.40 (80.49)	98.38 (85.00)	97.39 (82.75)
Konkan Wal 2	85.00 (67.89)	97.57 (84.07)	91.28 (75.98)
Hebbal 3	95.12 (77.93)	95.76 (79.64)	95.44 (78.78)
Arka Vijay	96.30 (80.79)	84.60 (67.16)	90.49 (73.98)
ACCW 113	90.75 (72.66)	90.36 (72.24)	90.55 (72.45)
ACCW 116	86.99 (69.08)	93.19 (75.88)	90.09 (72.48)
ACCW 147	89.12 (70.98)	87.00 (69.06)	88.06 (70.02)
ACCW 148	92.73 (74.94)	91.12 (73.08)	91.93 (74.01)
ACCW 165	93.75 (76.45)	89.49 (71.46)	91.62 (73.85)
ACCW 166	92.54 (74.41)	87.67 (70.31)	90.10 (72.36)
Mean	91.87 (74.79)	91.51 (74.54)	91.69 (74.66)
Range	85.00 (67.89) to 96.40 (80.49)	84.60 (67.16) to 98.38 (85.00)	88.06 (70.02) to 97.39 (82.75)
	<b>Genotype</b>	<b>Explant</b>	<b>Genotype X Explant</b>
S.E. ±	0.877	0.392	1.240
C.D. at 5% P	2.431	1.087	3.438

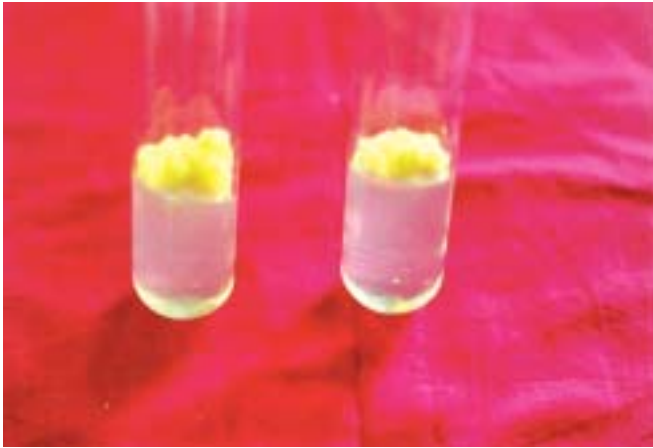
Figures in parenthesis indicate arc-sin values

**Table 2.** Genotypic variability in relation to growth regulator combination for callus induction on B<sub>5</sub> medium.

Genotypes	1	2	3	4	Mean
Konkan Wal 1	95.65 (79.42)	98.78 (86.82)	98.04 (84.36)	97.08 (80.38)	97.39 (82.75)
Konkan Wal 2	85.86 (69.40)	93.53 (79.78)	89.70 (72.49)	96.05 (82.26)	91.28 (75.98)
Hebbal 3	97.58 (82.03)	94.48 (76.75)	94.17 (76.55)	95.55 (78.79)	95.44 (78.78)
Arka Vijay	92.86 (76.66)	87.20 (69.69)	91.12 (75.02)	90.62 (74.52)	90.49 (73.98)
ACCW 113	94.33 (76.58)	86.63 (68.78)	90.25 (71.82)	91.00 (72.63)	90.55 (72.45)
ACCW 116	99.33 (89.25)	89.60 (71.73)	88.25 (70.16)	88.98 (70.77)	90.09 (72.48)
ACCW 147	87.91 (70.20)	86.72 (68.66)	89.43 (71.14)	88.19 (70.09)	88.06 (70.02)
ACCW 148	94.36 (76.91)	91.62 (73.48)	90.42 (72.52)	91.32 (73.13)	91.93 (74.01)
ACCW 165	94.20 (76.28)	89.87 (71.89)	90.77 (72.98)	91.65 (74.22)	91.62 (73.85)
ACCW 166	92.70 (74.90)	87.22 (69.93)	90.28 (72.20)	90.22 (72.39)	90.10 (72.36)
Mean	92.90 (75.96)	90.56 (73.75)	91.24 (74.02)	92.06 (74.92)	91.69 (74.66)
Range	85.86 (69.40) to 99.33 (89.25)	86.63 (68.78) to 98.78 (86.82)	88.25 (70.16) to 98.04 (84.36)	88.19 (70.09) to 97.08 (80.36)	88.06 (70.02) to 97.39 (87.75)
	<b>Genotype</b>		<b>GR</b>		<b>Genotype X GR.</b>
S.E. ±	0.877		0.555		1.754
C.D. at 5% P	2.431		1.538		4.862

Figures in parenthesis indicate arc-sin values

(1). 2.5 mg/l IAA + 5.0 mg/l BA, (2). 2.5 mg/l NAA + 5.0 mg/l BA, (3). 1.0 mg/l IAA + 10.0 mg/l BA, (4). 1.0 mg/l NAA + 10.0 mg/l BA



**Fig. 1.** Callus developed from stem explant on MS + 2.5 mg/l IAA + 5.0 mg/l BA

### Plantlet Regeneration

The data on percent response to regeneration in different genotypes using different explants are presented in Table 3. The maximum regeneration (89.06%) was recorded by Konkan Wal-1, while minimum regeneration

(71.87%) was recorded by ACCW-148. The mean response ranged from 71.87 to 89.06 percent. The genotypes Konkan Wal-1 and Arka Vijay were statistically at par with each other. The genotype Konkan Wal-1 is statistically significant over all the remaining genotypes.

The explants had highly significant effect on percent regeneration. The maximum regeneration (86.87%) was recorded by explant shoot-tip while minimum regeneration (68.56%) was recorded in cotyledonary node explant. The range of regeneration was from 78.12 to 100.00 percent and from 59.37 to 78.12 percent with shoot-tip and cotyledonary node explants, respectively. The shoot-tip explant was found better than cotyledonary node explant for plantlet regeneration (Kale 2004).

The relative ratio of auxin and cytokinin considerably influence the regeneration response (Sounder Raj *et al.* 1989). The morphogenic response of explants varies with combinations of growth regulators (Sounder Raj *et al.* 1991). The data regarding percent response to plantlet

**Table 3.** Genotypic variability in relation to explants for per cent plantlet regeneration on B<sub>5</sub> medium.

Genotypes	Shoot-tip	Cotyledonary node	Mean
Konkan Wal 1	100.00 (90.00)	78.12 (69.35)	89.06 (79.14)
Konkan Wal 2	90.62 (79.52)	71.87 (61.42)	81.25 (71.25)
Hebbal 3	84.37 (72.15)	68.75 (59.05)	76.56 (67.86)
Arka Vijay	93.75 (81.55)	78.12 (69.35)	85.94 (76.01)
ACCW 113	90.62 (79.52)	62.50 (52.41)	76.56 (67.86)
ACCW 116	78.12 (69.35)	66.67 (58.92)	72.40 (63.60)
ACCW 147	84.37 (72.15)	68.75 (59.05)	76.56 (67.86)
ACCW 148	78.12 (69.35)	65.62 (58.10)	71.87 (61.42)
ACCW 165	87.50 (73.10)	59.37 (55.65)	73.44 (65.47)
ACCW 166	81.25 (71.25)	65.79 (59.51)	73.52 (66.00)
Mean	86.87 (76.24)	68.56 (60.76)	77.72 (68.50)
Range	78.12 (69.35) to 100.00 (90.00)	59.37 (55.65) to 78.12 (69.35)	71.87 (61.42) to 89.06 (79.14)
	<b>Genotype</b>	<b>Explant</b>	<b>Genotype X Explant</b>
S.E. ±	2.342	1.047	3.312
C.D. at 5% P	6.492	2.903	9.181

Figures in parenthesis indicate arc-sin values

regeneration in different genotypes using different growth regulator combinations are presented in Table 4. The growth regulator combinations created variable response for per cent regeneration. The mean response ranged

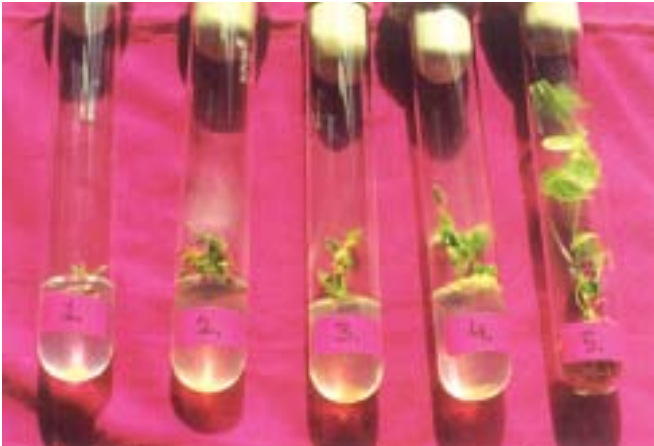
from 56.28 to 96.87 percent. The maximum (96.87%) and minimum (56.28%) regeneration was observed with 0.1 mg/l BA and 0.5 mg/l IAA + 0.5 mg/l BA, respectively. The plantlet regeneration from shoot-tip explant on MS + 0.1 mg/l BA is shown in fig.2.

**Table 4.** Genotypic variability in relation to growth regulator combinations for per cent pantlet regeneration on B<sub>5</sub> medium.

Genotypes	1	2	3	4	Mean
Konkan Wal 1	100.00 (90.00)	93.75 (81.55)	87.50 (73.10)	75.00 (60.00)	89.06 (79.14)
Konkan Wal 2	87.50 (73.10)	87.50 (73.10)	87.50 (73.10)	62.50 (52.41)	81.25 (71.25)
Hebbal 3	93.75 (81.55)	87.50 (73.10)	68.75 (59.05)	56.25 (48.79)	76.56 (67.86)
Arka Vijay	100.0 (90.00)	93.75 (81.55)	87.50 (73.10)	62.50 (52.41)	85.94 (76.01)
ACCW 113	93.75 (81.55)	93.75 (81.55)	65.50 (52.41)	56.25 (48.79)	76.56 (67.86)
ACCW 116	100.00 (90.00)	83.33 (68.10)	56.25 (48.79)	50.00 (45.00)	72.40 (63.60)
ACCW 147	100.00 (90.00)	93.75 (81.55)	68.75 (59.05)	43.75 (41.21)	76.56 (67.86)
ACCW 148	100.00 (90.00)	62.50 (52.41)	75.00 (60.00)	50.00 (45.00)	71.87 (61.42)
ACCW 165	93.75 (81.55)	75.00 (60.00)	65.50 (52.41)	62.50 (52.41)	73.44 (65.47)
ACCW 166	100.00 (90.00)	75.00 (60.00)	75.00 (60.00)	44.08 (41.51)	73.52 (66.00)
Mean	96.87 (85.96)	84.58 (72.98)	73.12 (63.99)	56.28 (51.06)	77.72 (68.50)
Range	87.50 (73.10) to 100.00 (90.00)	62.50 (52.41) to 93.75 (81.55)	56.25 (48.79) to 87.50 (73.10)	43.75 (41.21) to 75.00 (60.00)	71.87 (61.42) to 89.06 (79.14)
	<b>Genotype</b>		<b>GR.</b>		<b>Genotype X Gr.</b>
S.E. ±	2.342		1.481		4.684
C.D. at 5% P	6.492		4.104		12.984

Figures in parenthesis indicate arc-sin values

(1). 0.1 mg/l BA, (2). 0.2 mg/l BA, (3). 0.1 mg/l IAA + 0.1 mg/l BA, (4). 0.5 mg/l IAA + 0.5 mg/l BA



**Fig. 2. Plantlet regeneration from shoot tip explant on MS + 0.1 mg/l BA (stages of plantlet regeneration)**

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