# Effect of mutagen on callus induction and *in vitro* regeneration of soybean cultivar

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**Abstract:**

The effect of γ-irradiation on callus induction and regeneration in soybean cultivar 'Bragg' was studied. It was observed that callus induction was there in all the explants of control. Mean response for callus induction ranged from 47.30% (cotyledon, control) to 25.35% (leaf segments, 25kR). MS+2, 4-D 2mgl$^{-1}$ gave the best response for both leaf and cotyledon explants. There was significant effect of irradiation as the response of treated explants was low. The response for 20kR was better than 25kR. Mean callus weight ranged from 155mg (cotyledon, 25kR) to 273.23 mg (cotyledon, control). The callus obtained was of embryogenic, rhizogenic, and non-embryogenic morphotypes. Direct organogenesis was obtained from cotyledonary node, nodal segment, and shoot tips from both treated as well as control. Response for bud break was 71.48% in control, 62.32% in 20kR, and 57.20% in 25kR for cotyledonary nodes. The average number of days taken for bud initiation was 4.1 days in control, 5.7 days in 20kR, and 6.1 days in 25kR for cotyledonary nodes followed by nodal segments and shoot tips. Nodal segments gave the highest mean number of buds (9.92 in control, 8.65 in 20kR, and 6.24 in 25kR) followed by cotyledonary node. Mean number of buds obtained from shoot tip was very low (3.65 in control, 3.23 in 20 kR, and 3.05 in 25 kR). Treatment MS+BAP 5mgl$^{-1}$+IBA 0.1mgl$^{-1}$ was best. Root induction took place in ½ MS+IBA 0.8mgl$^{-1}$ or NAA 1.0mg mgl$^{-1}$ within 15-20 days.

**Introduction:**

To achieve desired improvements in soybean, breeders have used different breeding approaches. Most of these conventional approaches have their own limitations. Hence there is a need to supplement conventional breeding with plant biotechnology (Gahukar and Jambhule, 2000). Several techniques in tissue culture have been used effectively for crop improvement. There are reports on *in vitro* regeneration of soybean (Zheng *et al*, 1993; Hussian *et al*, 1996; Gai Junyi et al, 1996, 1997; Thome *et al*, 1995; Rajasekaran and Pellow, 1997; Settu and Kumari, 1998; and Ubanprasert *et al*, 1998) but reports on effect of irradiation on callus induction and regeneration in soybean are very scanty. Hence this investigation was attempted with the aim to study the effect of γ-irradiation on callus induction and regeneration in soybean cultivar 'Bragg'.

**Materials and methods:**

Seeds of soybean cultivar "Bragg" were given γ-rays treatment (20kR and 25kR).

7-10 days old *in vitro* germinated seedlings of both treated and control were used to collect explants like cotyledon, cotyledonary node, nodal segment, leaf segment, and shoot tips. Different combinations of growth regulators (BAP, IAA, IBA, and 2,4-D) with MS basal medium were used to study their effect on the various explants. Explants inoculated with the culture from germination to callus induction, regeneration, and rooting were maintained at 25 ± 2°C temperature and a photoperiod of 16 hrs of light and 8 hrs of darkness. Observations were recorded for response on callus induction, number of days to callus induction, callus fresh weight, callus morphology, response to shoot differentiation, days for shoot differentiation, number of shoot buds per culture, response on root differentiation, days for root differentiation (for both direct and indirect pathway of regeneration). The experiment for all aspects was conducted in CRBD and the mean values of 5 aliquots were used in duplex for analysis.

**Results and Discussion:**

It was observed that callus induction occurred in all the explants of control. However in treated explants some of the treatment combinations failed to induce callus. Mean response for callus induction ranged from 47.30% (cotyledon, control) to 25.35% (leaf

segments, 25kR) (Table.1). MS+2, 4-D 2mgl$^{-1}$ gave the best response for both leaf and cotyledon explants. There was significant effect of irradiation as the response of treated explants was low. Gahukar and Jambhule, 2000, also found similar type of decrease in callus obtained with increased dose of gamma rays and EMS in sugarcane. The response for 20kR was better than 25kR. Mean callus weight ranged from 155mg (cotyledon, 25kR) to 273.23 mg (cotyledon, control). Similar decrease in callus fresh weight was observed by Reddy *et al*, 1987, in castor bean and by Singh and Singh, 1993, in sugarcane with the increase in the dose of irradiation. Higher doses of gamma irradiation caused considerable tissue damage, which perhaps in turn leads to reduction in callus fresh weight. The callus obtained was of embryogenic, rhizogenic, and non-embryogenic morphotypes. Rhizogenic callus failed to regenerate into shoot, owing to root differentiation and proliferation. Direct organogenesis was obtained from cotyledonary node, nodal segment, and shoot tips from both treated as well as control. Response for bud break was 71.48% in control, 62.32% in 20kR, and 57.20% in 25kR for cotyledonary nodes (Table.2). The average number of days taken for bud initiation was 4.1 days in control, 5.7 days in 20kR and 6.1 days in 25kR for cotyledonary nodes followed by nodal segments and shoot tips. Treated explants took more days than the controls for bud initiation. Nodal segments gave the highest mean number of buds (9.92 in control, 8.65 in 20kR, and 6.24 in 25kR) followed by cotyledonary node. Mean number of buds obtained from shoot tip was very low (3.65 in control, 3.23 in 20kR, and 3.05 in 25kR). Treatment MS+BAP 5mgl$^{-1}$+IBA 0.1mgl$^{-1}$ was best. Root induction took place in ½ MS+IBA 0.8mgl$^{-1}$ or NAA 1.0mg mgl$^{-1}$ within 15-20 days. The influence of irradiation was on the lower side far all the aspects studied. This influence was linearly related to increase in concentration of gamma rays dose.

**Conclusion:**

Considering the frequency of regeneration both direct and indirect through callus and the effect of irradiation, an alternate method for obtaining additional variants can be followed to generate large quantity of callus for cell suspension culture on specific media, exerting selection pressure to screen out variant cells, which then can be allowed to regenerate to give somaclonal variants.

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**Table1. Treatments in which callus induction was found good**

| | | Cotyledon | | | Leaf segment | | |
|---|---|---|---|---|---|---|---|
| **Sr no.** | **Treatment ($mgl^{-1}$)** | **Control** | **20 kR** | **25 kR** | **Control** | **20 kR** | **25 kR** |
| | **% Response to callusing** | | | | | | |
| 1. | MS + IAA 2.0 | 70.0 | 42.1 | 40.0 | 78.94 | 52.63 | 47.36 |
| 2. | MS + 2,4-D 2.0 | 90.0 | 57.89 | 50.0 | 84.21 | 50.0 | 45.0 |
| 3. | MS + BAP 0.5+ NAA 2.0 | 84.21 | 50.0 | 45.0 | 81.25 | 47.05 | 40.0 |
| | SE (d ) | 1.30 | 0.68 | 0.84 | 0.69 | 0.53 | 0.77 |
| | CD ( 5% ) | 2.68 | 1.43 | 1.76 | 1.42 | 1.11 | 1.60 |
| | CD ( 1% ) | 3.62 | 1.95 | 2.41 | 1.92 | 1.51 | 2.18 |
| | **Days to callus induction** | | | | | | |
| 1. | MS + IAA 2.0 | 9.1 | 11.0 | 11.1 | 3.9 | 6.3 | 6.5 |
| 2. | MS + 2,4-D 2.0 | 7.0 | 9.4 | 9.7 | 3.1 | 5.5 | 5.6 |
| 3. | MS + BAP 0.5+ NAA 2.0 | 7.7 | 9.7 | 9.8 | 3.3 | 5.6 | 5.9 |
| | SE (d ) | 0.11 | 0.14 | 0.12 | 0.12 | 0.12 | 0.14 |
| | CD ( 5% ) | 0.23 | 0.31 | 0.27 | 0.26 | 0.25 | 0.29 |
| | CD ( 1% ) | 0.31 | 0.42 | 0.37 | 0.35 | 0.35 | 0.40 |
| | **Callus weight ( g )** | | | | | | |
| 1. | MS + IAA 2.0 | 422.04 | 163.3 | 151.2 | 372 | 181.4 | 162.6 |
| 2. | MS + 2,4-D 2.0 | 538.7 | 195.1 | 182.9 | 444.5 | 199.6 | 184.1 |
| 3. | MS + BAP 0.5+ NAA 2.0 | 478.71 | 183 | 171.4 | 349.3 | 181.7 | 171.4 |
| | SE (d ) | 7.22 | 1.64 | 0.81 | 12.46 | 1.95 | 1.27 |
| | CD ( 5% ) | 14.79 | 3.44 | 1.70 | 25.54 | 4.05 | 2.63 |
| | CD ( 1% ) | 19.96 | 4.71 | 2.32 | 34.46 | 5.51 | 3.58 |

**Table 2. Effect of different treatments on percentage response to bud break**

| | | Cotyledonary node | | | Nodal segment | | | Shoot tip | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Tr.No.** | **Treatment ($mgl^{-1}$)** | **Control** | **20 kR** | **25 kR** | **Control** | **20 kR** | **25 kR** | **Control** | **20 kR** | **25 kR** |
| T1 | MS+BAP 0.1 | 45.0 | 42.10 | 35.0 | 44.44 | 40.0 | 36.84 | 38.88 | 33.33 | 29.41 |
| T2 | 1.5 | 50.0 | 44.44 | 38.88 | 52.94 | 44.44 | 41.17 | 50.0 | 41.17 | 33.33 |
| T3 | 2.0 | 68.42 | 52.63 | 50.0 | 65.0 | 52.63 | 45.0 | 56.25 | 47.36 | 41.17 |
| T4 | 2.5 | 57.89 | 50.0 | 47.36 | 57.89 | 47.36 | 38.88 | 50.0 | 42.10 | 35.0 |
| | MS+BAP+IBA | | | | | | | | | |
| T5 | 1+0.05 | 61.11 | 52.63 | 50.0 | 63.15 | 55.55 | 47.05 | 57.89 | 43.75 | 38.88 |
| T6 | 2+0.05 | 70.0 | 63.15 | 55.55 | 72.22 | 57.89 | 50.0 | 63.15 | 50.0 | 44.44 |
| T7 | 3+0.05 | 84.21 | 70.0 | 63.15 | 80.0 | 67.70 | 61.11 | 72.22 | 57.89 | 52.94 |
| T8 | 4+0.05 | 75.0 | 66.66 | 60.0 | 76.47 | 60.0 | 52.63 | 70.58 | 55.55 | 47.36 |
| | MS+BAP+IBA | | | | | | | | | |
| T9 | 2+0.1 | 73.68 | 68.42 | 52.94 | 68.75 | 58.82 | 52.94 | 58.82 | 50.0 | 41.17 |
| T10 | 3+0.1 | 77.77 | 70.58 | 55.55 | 73.36 | 66.66 | 57.89 | 66.66 | 58.82 | 47.36 |
| T11 | 4+0.1 | 80.0 | 77.77 | 68.42 | 77.77 | 70.0 | 64.70 | 72.22 | 63.15 | 55.55 |
| T12 | 5+0.1 | 90.0 | 85.0 | 78.94 | 84.21 | 73.68 | 70.0 | 80.0 | 72.22 | 62.50 |
| | MS+BAP+IBA | | | | | | | | | |
| T13 | 0.2+0.5 | 52.94 | 43.75 | 40.0 | 52.63 | 44.44 | 47.05 | 47.05 | 40.0 | 35.29 |
| T14 | 0.4+0.5 | 63.15 | 50.0 | 44.44 | 60.0 | 50.0 | 50.0 | 55.55 | 43.75 | 38.88 |
| T15 | 0.8+0.5 | 72.22 | 60.0 | 55.55 | 66.66 | 55.0 | 61.11 | 58.82 | 50.0 | 41.11 |
| T16 | 1.0+0.5 | 75.0 | 68.42 | 43.15 | 70.58 | 61.11 | 52.63 | 63.15 | 55.0 | 50.0 |
| | MS+BAP+NAA | | | | | | | | | |
| T17 | 2+0.1 | 75.0 | 72.22 | 64.7 | 75.0 | 66.66 | 57.89 | 63.15 | 55.55 | 47.05 |
| T18 | 3+0.1 | 80.0 | 73.68 | 66.66 | 81.25 | 72.22 | 65.0 | 72.22 | 64.70 | 57.89 |
| T19 | 4+0.1 | 83.33 | 80.0 | 70.0 | 83.33 | 76.47 | 70.58 | 78.94 | 70.0 | 63.15 |
| T20 | 5+0.1 | 95.0 | 85.0 | 83.33 | 89.47 | 80.0 | 75.0 | 83.33 | 77.77 | 66.66 |
| | **GM** | 71.48 | 62.32 | 57.20 | 69.75 | 59.88 | 53.65 | 62.94 | 53.60 | 46.45 |
| | **SE ( d )** | 0.33 | 0.78 | 0.89 | 0.52 | 0.56 | 0.48 | 0.42 | 0.64 | 0.47 |
| | **CD 5%** | 0.69 | 1.63 | 1.85 | 1.08 | 1.17 | 1.01 | 0.88 | 1.35 | 0.98 |
| | **CD 1%** | 0.94 | 2.23 | 2.53 | 1.48 | 1.60 | 1.38 | 1.20 | 1.84 | 1.34 |