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# Callusing from wheat root explants: Effect of different developmental stages and gamma irradiation doses

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**Abstract**
Root tips of about 5-6 mm length excised from 3,4 and 5 days old seedings of nontreated seeds were cultured on $B_5$ medium + 10mg/12, 4-D. Callus initiation took place after 32 days in 3 days old root tips, 26 days in 4 days old and 23 days in 5 days old root tips. Callus induction was significantly highest in 3 days old root tips (34.6%) among 4 days old (13.7%) and 5 days old root tips (25.1%) 30KR and 35KR gamma irradiated seeds derived root tips of only 3 days old seedlings were also cultured on the same medium. Callus initiation took place within 4 days and callusing was 72.3 percent in 30KR and 71.9 percent in 35KR root tips. Shoot regeneration was negligible from 3, 4 and 5 days old root tips of nontreated seed. While large number of green spots with profused network of roots were formed on regeneration media. However 30 KR and 35 KR root tips calli showed shoot regeneration but frequency was low.

**Keywords:** Wheat, root tip, callusing and gamma irradiation

## Introduction
In recent years advances in the field of plant tissue culture and molecular biology have led to "speculation that techniques involving the manipulation of (issues in-vitro may he directly applicable to the genetic alteration and improvement of crop plants for which production of sufficient callus is prerequisite. In comparison with rice, wheat remains a species needing additional research regarding genetic transformation. Most reports on the establishment of wheat callus and its subsequent differentiation to whole plants have used immature embryos (Hakam *et al*. 2014) [6] immature inflorescences (Yadava et.al. 2002) [11] and mature embryos (Rashid, 2009) [10] as explants. In wheat, root tips have proved difficult to culture *In-vitro*. So very few reports are available on *In-vitro* culture from root tips in wheat. Among the explants, young seedlings derived root tips are the most easily available explants. Since they can be grown *In-vitro* and a short term, frequent supply of explants can be provided. It is important to appreciate that the quantity of primary callus which obtained from root explants is significant, as 4-5 roots are formed from each seed and each of these roots can give a large number of primary calli (more than 100 from a single root) as compared to other explants. This paper described that callus initiation and frequency of callusing is significantly effected with age of explants as well as different doses of gamma irradiation which was the objective of this study.

## Materials and Methods
Nontreated and gamma irradiated seeds with 30K.R and 35 KR doses were soaked overnic.ht, after washing with tween 80 and surface sterilization with 0.1% $HgCl_2$ (5 min.). The sterilized seeds after washing with sterile distilled water (4 times) were germinated on solidified germinating medium (20% sucrose w/v and 0.8% agar w/v only). Root tips excised from 3,4 and 5 days old seedlings were cultured on $B_5$ medium +10mg/l 2,4-D. However 3 days old root tips were more responsive for callusing, so further only: 3 days old root tips of 30 KR and 35KR gamma irradiated seeds were used. Callus remained on the same medium for about 40 days and transferred to regeneration media. Various combinations of different growth regulators at different concentration were used in MS medium for regeneration.
1. MS+1.0mg/l BAP+0.2mg/l NAA
2. MS+0.5mg/12, 4-D+0.2mg/l NAA
3. MS+0.lmg/l NAA
4. MS+1.0mg/1 BAP+0.2mg/l IAA
5. MS+0.2mg/l NAA
6. MS medium without growth regulator.

Glutamine at different level (l-5mg/l) were also used for regeneration in MS medium.

**Culture conditions**

All the media were adjusted to pH 5.8+1 before autoclaving. Cultures were kept in dark at 25+l°C for callusing and in a 16h photoperiod (white flurescent light) for regeneration.

**Statistical Analysis**

After angular transformation, the replicated data were analysed by using Completely Randomized Design (CRD) and critical differences were calculated.

**Results and Discussion**

It is found that detached roots of young seedlings (3,4 and 5 days old) of six different wheat genotypes when cultured on $B_5$ +10mg/ 1 2,4-D medium, callus initiation took place after 32 days in 3 days old root tips (34.6%), 26 days in 4 days old (13.7%) and 23 days in 5 days old root tips (25.1 %). Significantly highest callusing and callus growth (by visual observation) was observed in 3 days old root tips. Callusing percentage range was 0.5 to 68.3 in 3 days and 0.5 to 79.7 in 4 days old and 0.5 to 83.9 in 5 days old root tips. In all the cases WH 542 exhibited highest callusing and callus growth. Significant differences were also observed among the cultivars (Table 2) Root callus was white and watery (Fig 1) Roots formed callus from their basal portion to all through its length and higher was near the root tip portions as compared to distal root tip portion (Fig 1). It is noteworthy that the callus initiation, from 30 KR and 35 KR gamma irradiation seeds derived 3 days old root tips was earlier (within 4 days after inoculation) than nontreated ones. Callusing percentage was 72.3 in 30 KR root tips and 71.9 in 35 KR roots tips and highest was observed in Raj 3765 (83.3% in 30KR and 83.0% in 35KR root tips). Callus growth was best in UP 2338 followed by WH 542 (visual observation) in both. Calli were remained on the same medium for about 5-6 weeks. Calii pieces, developed from all type of root tips were transferred to various six regeneration media. It is seen that most of root calli developed roots only but large number of green spots (70%) were also formed. Callus exhibited no growth and generally browned if maintained for periods of more than 3 weeks. However roots continue to grown in the medium. Roots calli which had been selected and the propagated for a further 3 weeks on other regeneration media also did not differentiated into shoot at any of six media (tested in present study) and developed a dense network of very fine root hairs (Fig 2). Glutamine at different levels (0-5 mg/1) also failed to induce shoots as root calli had completely lost their capacity to form shoots. Thus primary root calli formed roots because the callus probably contained only root meristems. The ability of the callus to differentiate will decline after progressive subcultures on different regeneration media for two possible reason. Firstly, the integrity of the meristem may have been destroyed during rapid cell proliferation. Secondly, root calli gave a highly synchronized response elicited by application of different growth regulators as calli were transferred continuously on different media for long periods for regeneration. As cultures for regeneration were maintained in the light, the developed roots had a light green tinge. Similar was reported by Chin and Scott (1977) [3] but they reported poor callusing in comparison to present results as well as Butenko-(1986) [2] reported only 10-15% callusing from root explains on medium containing kinetin and 2,4-D on the 8th day.

Root tips of 3 days old seedling showed significantly highest callusing among 4 and 5 days old root tips. It exhibited that the formation of callus is an age-dependent response. Similar findings, I have earlier been documented for shoot tip explants in wheat, Sharma *et al.* (2003) [9]. None of such observations have so far been reported for root explants in wheat. However, the quantity of primary calli obtained from root explants is significantly high.

It is left, to be determined how competent the root derived calli are in uptake and integration of the foreign gene in wheat as compared to calli obtained from other explants, Mukhopadhyay *et al.* (1997) [7] presently investigating utility of root derived calli in genetic transformation experiments in rice. In present study gamma irradiation treatments highly enhanced the callus induction with earliest callus initiation and good callus growth of root tip explants. Regeneration was also observed from these calli (Fig. 3a, b). While frequency was very low (data not presented) Gao *et al.* (1986) [5] and Abdrabou (1992) [1] also reported positive effect of gamma irradiation treatment on callusing and regeneration in immature embryo of wheat. However root callus may also be served as an ideal source for originating cell suspension cultures. The present results revealed callusing up to 83.9 percent which is quite high. The use of root tip explants as an alternative source for initiating callus culture and further tissue culture studies including induction of somaclonal variation is also suggested in wheat. On the whole, present study provide new scientific information and improvement in terms of technique.

**Table 1:** Analysis of variance and the meansquares for callus induction from root explants of wheat

| S.O.V. | D.F. | M.S |
|---|---|---|
| Factor A | 04 | 7539.58 |
| Factor B | 05 | 2277.37 |
| Factor AxB | 20 | 1002.36 |
| Error | 60 | 11.12 |

A - Treatment
B - Genotype

**Table 2:** Effect of different developmental stages and gamma irradiation on callus induction in root tip of different wheat genotypes

| Genotype | Root tip callusing (%) | | | | |
|---|---|---|---|---|---|
| | 3 days | 4 days | 5 days | 3 days (30 KR) | 3 days (35 KR) |
| DI 9 | 51.8 (46.32) | 0.50 (4.05) | 0.50 (4.05) | 73.9 (59.46) | 74.9 (60.09) |
| UP 2338 | 66.6 (55.09) | 0.50 (4.05) | 0.50 (4.05) | 79.3 (63.03) | 76.6 (61.15) |
| Raj 3765 | 0.50 (4.05) | 0.50 (4.05) | 64.9 (53.86) | 83.3 (66.37) | 83.0 (65.79) |
| WH 147 | 0.50 (4.05) | 0.50 (4.05) | 0.50 (4.05) | 64.6 (53.64) | 59.7 (50.65) |
| PBW 343 | 20.00 (26.81) | 0.50 (4.05) | 0.50 (4.05) | 67.4 (55.21) | 68.7 (56.07) |
| WH 542 | 68.3 (56.16) | 79.9 (63.75) | 83.9 (66.99) | 65.6 (54.12) | 68.7 (56.07) |

| Total Mean | 34.62 (32.08) | 13.70 (14.00) | 25.13 (22.84) | 72.35 (58.64) | 71.9 (58.30) |
|---|---|---|---|---|---|
| C.D. | A | = | 2.22 | | |
| | B | = | 2.44 | | |
| | AxB | = | 5.45 | | |

Figures in parentheses are angular transformed values

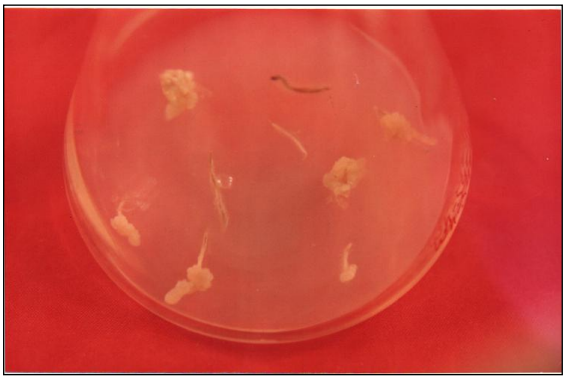


**Fig 1:** Callus induction from root tip of wheat.

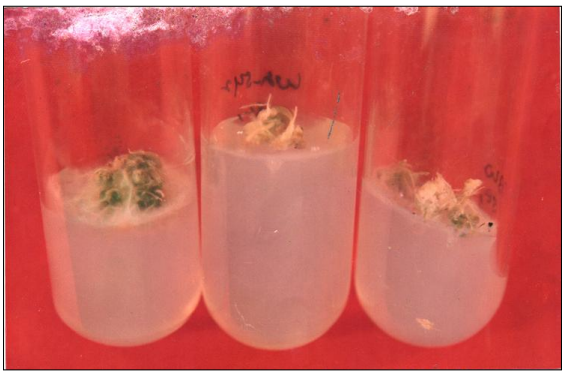
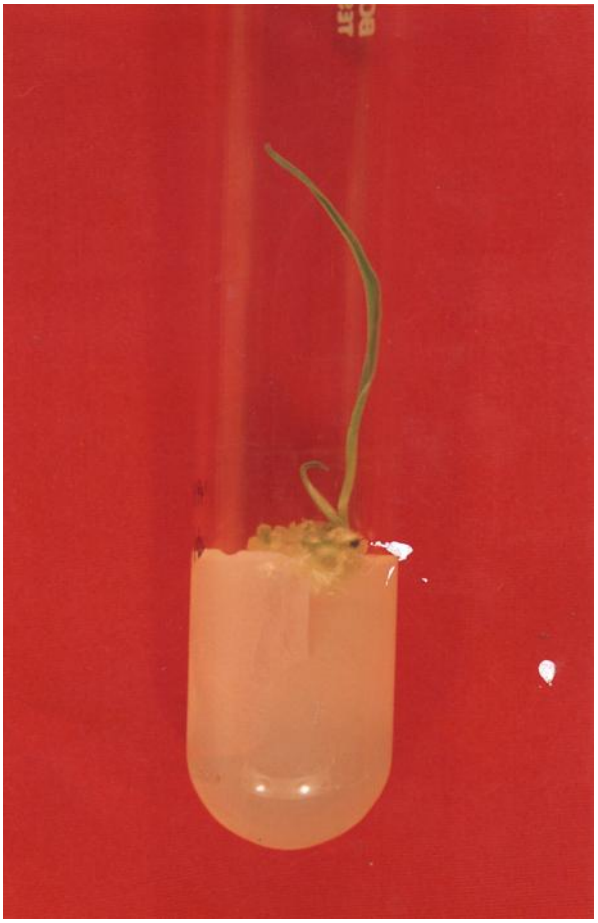


**Fig 2:** Dense network of very fine root hairs and green spots formation from root tip calli on regeneration.

**(a)**



**(b)**

**Fig 3:** Shoot initiation (a) and shoot formation (b) from gamma irradiated root tip calli.

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