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**Research Article**

# Callus induction and plant regeneration from immature embryos of spring wheat varieties *(Triticum aestivum* L.) under different concentrations of growth regulators through tissue culture technique

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**Abstract**

This experiment was conducted at Nuclear Institute of Agriculture, Tandojam and Sindh Agriculture University Tandojam. Various concentrations of growth regulators comprising 2, 4-D (2, 4-Dichlorophenoxyacetic acid), Picloram, (Pic), 1-Naphthaleneacetic acid (NAA), Kinetin (Kin), Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA) and 6-Benzylaminopurine (BAP) were used in this study. The observations recorded were weight of proliferation bottle$^{-1}$, weight of callus bottle$^{-1}$, number of plantlets regeneration bottle$^{-1}$ and number of roots plant$^{-1}$. The result of experiment indicated that maximum proliferation and callus induction were recorded with concentration of MS + 2 mg /L 2, 4-D, followed with MS + 2 mg /LPicloram, while maximum plant regeneration with concentration of MS + IAA 6.0 mg /L + 6.0 mg /L Kin, followed by MS + IAA 5.0 mg /L + 4.0 mg /L Kin. However, maximum roots were recorded with MS + IBA 2.0 mg /L + 30 g/L, followed by under concentration of MS + IBA 2.0 mg /L + 20 g/L. It was concludedthat2, 4-D was best for proliferation and callus induction, while the concentration of MS + IAA 5.0 mg /L + 4.0 mg /L Kin+ 30 g/L for plant regeneration and MS + IBA 2.0 mg /L + 30 g/L were found best concentration for root induction. This research is reliable for the different effects of the phytohormones and the production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds development of somaclone plants.

**Key words:** Wheat varieties; Callus induction; Regeneration; Rooting

## Introduction

Food is the primary concern and the most important of living organisms. Being an important crop, Wheat (Triticumaestivum L.) is to enhance the agronomic attributes and biotic or abiotic stress by means of genetic engineering. Wheat is one of the first domesticated food crops and the main staple food for the past 8,000 years. Wheat is the most demanded food grain production leading all crops, including rice, corn and potatoes. Energy and proteins are considered to be common source provided from wheat to the World's population. Bread wheat is one of the major staple sustenance products developed around the world; that covers very nearly 17% of aggregate range of world under cultivation and there is dynamic increment in wheat yield and interest all through the world for expanding human populace development [1].It has been assessed that a noteworthy increment of more than 40% in wheat yield is needed by 2020 to meet growing demand of human populace [8]

Conventional plant reproducing methods in spite of the fact that have been rehearsed effectively since 1960s for the production of enhanced wheat varieties however can possibly meet such an incredible test because of accessibility of restricted quality pool [8].Genetic change empowers the presentation of novel genes specifically into by regional standards adjusted genotypes to make new genetically modified varieties utilizing tissue culture method as their base foundation and through this procedure to develop a whole plant that can be regenerated from isolated cells whereas numerous isolated have been produced, however in wheat they all are that much genotypic dependent [1].Wheat growth and yield is being affected by factors consisting temperature, moisture, soil and light [2]. Drought and salt stress are the most common environmental constraints which caused a significant decline in growth, development and yield on current agricultural land. In addition, they are great problems in wheat cultivation in arid and semi-arid regions [3]. Rehman*et al.*, 2008, conducted an experiment establishing cell suspension cultures from cultured immature embryos of wheat cultivars [4]. Trials were conducted on embryos (mature and immature) cultured on medium MS supplemented with 2, 4, D. Low frequency was observed to be in mature embryos whereas high regeneration was to be found in embryos which were immature [5]. It has been reported that after adding BAP in media with 2, 4-D, high frequencies of embryo genic callus was observed in immature inflorescences [6]. Plant transformation is considered to be one of the key steps in regeneration [2]. Many have reported that callus culture derived from wheat plants have been regenerated from different plants having low rate of green plant regeneration [7]. Some have reported that cells are chosen and preferred, maintaining an adequate level of 2, 4-D helps continue embryo genetic nature of culture because of the perpetual division of the embryo genic cells and dynamic meristematic zones framed in proliferating tissues. Immature embryos and immature inflorescences results most extreme recurrence of recovery of plants in *vitro* [6]. When embryos were compared immature to mature embryos, the former we're a better choice for regeneration. Immature embryos are extremely regenerative because it contains embryo genictotipotent cell [8]. Type of grains, especially wheat, dicots lagged in its response in vitro techniques and remains relatively dependent on the genotype [1]. Needs of biotechnology in crop development is successful when whole plant tissues from transgenic selection is regenerated. Therefore, it is widely studied with respect to regeneration of plants from in vitro culture. The purpose of this research was to screen efficient *In vitro* plant regeneration from immature wheat embryos of two cultivars varieties Khirman and Sarsabz.

## Materials and methods

The research was carried out as joint venture among Tissue Culture Laboratory,

Plant Breeding and Genetics Division, Nuclear Institute of Agriculture Tandojam and Department of Biotechnology, Sindh Agriculture University Tandojam. Two varieties of wheat genotype viz. Khirman and Sarsabz were selected for the conducted research. Spikes were obtained from (Experimental Farm of Nuclear Institute of Agriculture Tandojam, Pakistan). Spikes were sterilized with alcohol for one minute. For callus induction, immature embryos were removed with a scalpel from imbibed seeds. For callus induction, the effects of two induction media were compared. Immature embryos with scutellum were kept upwards on a solid agar medium and cultured for 14 days at 25 ± 2ºC under a 16 h photoperiod. The basal culture media consisted of the mineral saltssupplemented with 2 mg/L 2, 4-D, NAA and Picloram. Both media contained 30 g/L sucrose and was attuned to pH level of 5.7, solidified with 8 g/L agar and then kept for 15 minutes at 121ºC. Calli were transferred to MS medium (MS with full strength macronutrients) with growth regulators and cultured at 25 ± 2ºC in a 16h/8h light/dark cycle for 3-4 weeks for shoot and root initiation. When roots and shoots were established, young plants were grown in bottles containing the same medium. Weight of callus bottle$^{-1}$(g), Weight of callus proliferation bottle$^{-1}$(g), Number of plantlets bottle$^{-1}$ and Number of roots plant$^{-1}$ were the parameters kept under observation. Wheat varieties with different concentrations (Factor A and B) with different treatments as mentioned below. The design was Randomized Complete Block Design (factorial), laid outreplicated thrice.

**Factor (A)**

Wheat Varieties =Khirman ($V_1$) & Sarsabz ($V_2$)

**Factor (B)**

Concentrations (2mg/L) = 2,4-D, Picloram & NAA

**Proliferation and callus induction**

MS (Control)
MS + 2 mg/L 2, 4-D (PCI-1)
MS + 2 mg/L Picloram(PCI-2)
MS + 2 mg/L NAA(PCI-3)

**Plantlets of plantlets**

MS + IAA 6.0 mg /L + 6.0 mg /L Kin + 25 g /L(PP-1)
MS + IAA 5.0 mg /L + 5.0 mg /L Kin + 25 g /L(PP-2)
MS + IAA 2.0 mg /L, IBA 2.0 mg /L + 2.0 mg /L BAP + 25 g /L(PP-3)
MS + IAA 2.5 mg /L, IBA 2.5 mg /L + 2.5 mg /L BAP + 25 g /L(PP-4)
MS + IAA 3.0 mg /L, IBA 3.0 mg /L + 3.0 mg /L BAP + 25 g /L(PP-5)

**Root induction**

MS (Control) + sugar 30 g /L
MS + IBA 2.0 mg /L + sugar 10 g /L (RTI-1)
MS + IBA 2.0 mg /L + sugar 20 g /L (RTI-2)
MS + IBA 2.0 mg /L + sugar 30 g /L (RTI-3)

**Statistical analysis**

The experimental data were recorded and subjected to factorial design of analysis of variance (ANOVA) under linear models of statistics to observe statistical differences among different traits of wheat using computer program, Student Edition of Statistix (SWX), Version 8.1 (Analytical Software, 2005). Further least significant difference (LSD) test was also applied to test the level of significance among different combination means **[10]**.

**Results and discussions**

**Proliferation in wheat varieties**

These results of proliferation indicated that varieties, concentrations were highly significant and their interactions were non-significant in (Table and Figure 1).The mean of varieties showed that maximum proliferation was observed (1.71, 1.99 g) respectively in Sarsabz and Khirman. The maximum proliferation was observed (2.39 g) under concentration of PCI-1, followed by (2.14 g) with PCI-2 and minimum were observed under control (1.05 g).The interactive effect of varieties x concentrations indicated that maximum proliferation were observed (2.26 and 2.52 g) in Sarsabz and Khirman under the concentration of PCI-1, followed by (1.99

and 2.30 g) with PCI-2 and minimum proliferation was recorded (0.95 and 1.14 g) under control. The results showed that 2, 4-D produced more proliferation as compared with Picloram and NAA. The results agreed with [11, 6] that MS + 2 mg /L 2, 4-D produced more proliferation as compared with Picloram and NAA.

**Table 1. Proliferation (g) supplemented with different concentrations of growth regulators**

| Concentrations | Varieties | | Mean |
|---|---|---|---|
| | Sarsabz | Khirman | |
| MS (Control) | 0.95 f | 1.14 e | 1.05 d |
| PCI-1 | 2.26 b | 2.52 a | 2.39 a |
| PCI-2 | 1.99 c | 2.30 b | 2.14 b |
| PCI-3 | 1.66 d | 2.00 c | 1.83 c |
| Mean | 1.71 b | 1.99 a | |

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replications | 4 | 0.1389 | 0.03473 | | |
| Varieties | 1 | 0.7563 | 0.75625 | 56.20 | 0.0000 |
| Treatments | 3 | 10.2283 | 3.40942 | 253.36 | 0.0000 |
| V x T | 3 | 0.0327 | 0.01090 | 0.81 | 0.4992 |
| Error | 28 | 0.3768 | 0.01346 | | |
| Total | 39 | 11.5329 | | | |

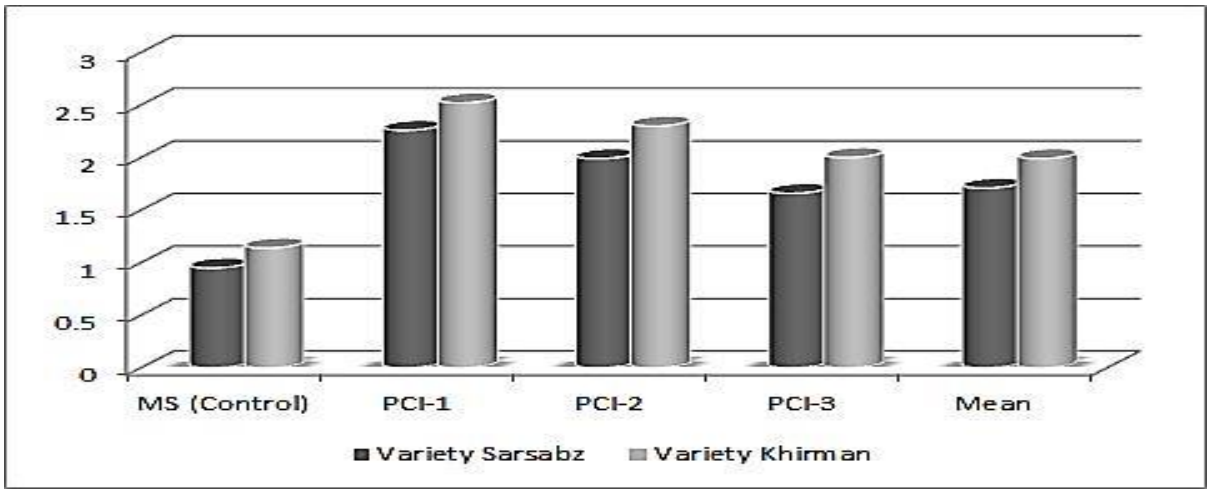


**Figure 1. Proliferation in wheat**

## Callus induction

The results of statistical analysis of variance showed that varieties, concentrations and their interactions were highly were significant and are presented in (Figure and Table 2). There results of varieties indicated that maximum callus induction (2.13 and 2.51 g) was achieved in Sarsabz and Khirman. The maximum callus induction was recorded PCI-1 (3.08 g), followed by (2.75 g) under concentration of PCI-2and minimum (1.37 g) was obtained in control. The results of their interactions indicated that maximum callus induction was observed (2.95 and 3.21 g), followed by (2.48 and 3.02 g) PCI-2 and minimum callus was observed under control in Sarsabz and Khirman respectively. The results supported by [6] and [11] that the MS + 2 mg /L 2, 4-D produced highest rate of callus induction was obtained as compared with Picloram and NAA.

**Table 2. Callus induction (g) supplemented with different concentrations of growth regulators**

| Concentrations | Varieties | | Mean |
|---|---|---|---|
| | Sarsabz | Khirman | |
| MS (Control) | 1.17 g | 1.57 f | 1.37 d |
| PCI-1 | 2.95 b | 3.21 a | 3.08 a |
| PCI-2 | 2.48 c | 3.02 b | 2.75 b |
| PCI-3 | 1.94 e | 2.24 d | 2.09 c |
| Mean | 2.13 b | 2.51 a | |

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replications | 4 | 0.0323 | 0.00808 | | |
| Varieties | 1 | 1.4063 | 1.40625 | 76.65 | 0.0000 |
| Treatments | 3 | 17.1198 | 5.70659 | 311.04 | 0.0000 |
| V x T | 3 | 0.1189 | 0.03964 | 2.16 | 0.1150 |
| Error | 28 | 0.5137 | 0.01835 | | |
| Total | 39 | 19.1910 | | | |

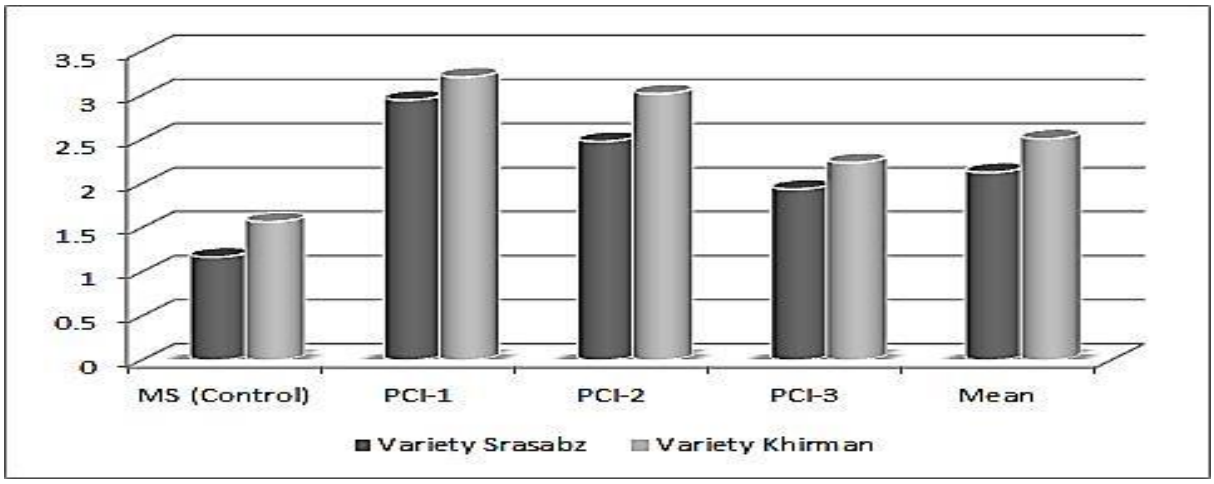


**Figure 2. Callus induction in wheat varieties**

**Regeneration of plantlets**

The results of statistical analysis of variance showed that varieties, concentrations and their interactions were highly were significant and data are presented in (Table and Figure 3). The result of varieties showed that maximum plantlets were regenerated (3.88 and 4.72) in Sarsabz and Khirman varieties. The results of different concentrations indicated that maximum plantlets were regenerated (6.00) in PP-4, followed by (5.30) under concentration of PP-3 and minimum plantlets was regenerated (2.20) under control. The interactions of varieties x concentration indicated that maximum plantlets were regenerated (5.40 and 6.40) under concentration of PP-4, followed by (4.80 and 5.80) under concentration of PP-3, while the minimum plantlets were regenerated (1.80 and 2.60) under concentration of PP-1 in Sarsabz and Khirman varieties. The results agreed with [12].

**Table 3.Regeneration of plantlets supplemented with different concentrations of growth regulators**

| Concentrations | Varieties | | Mean |
|---|---|---|---|
| | Sarsabz | Khirman | |
| PP-1 | 1.80 g | 2.60 fg | 2.20 d |
| PP-2 | 4.00 c-e | 5.00 bc | 4.50 b |

| | | | |
|---|---|---|---|
| PP-3 | 4.80 b-d | 5.80 ab | 5.30 a |
| PP-4 | 5.60 ab | 6.40 a | 6.00 a |
| PP-5 | 3.20 ef | 3.80 de | 3.50 c |
| Mean | 3.88 b | 4.72 a | |

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replications | 4 | 2.600 | 0.6500 | | |
| Varieties | 4 | 8.820 | 8.8200 | 11.76 | 0.0015 |
| Treatments | 4 | 89.800 | 22.4500 | 29.93 | 0.0000 |
| V x T | 4 | 0.280 | 0.0700 | 0.09 | 0.9840 |
| Error | 36 | 27.000 | 0.7500 | | |
| Total | 49 | 128.500 | | | |

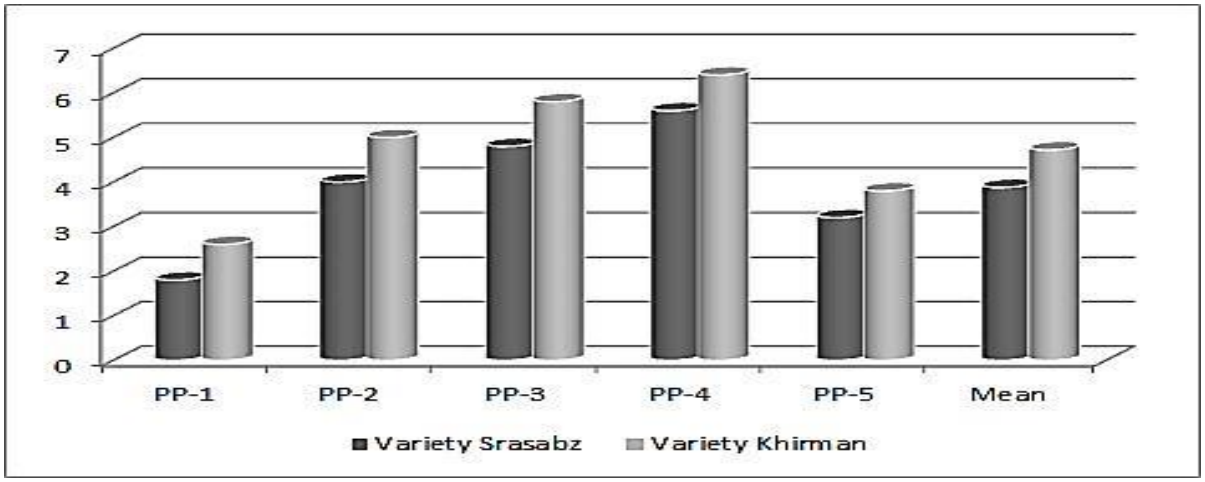


**Figure 3. Regeneration of plantlets in wheat varieties**

**Root induction**

The results of analysis of variance showed that varieties, concentrations and their interactions were highly were significant and data are presented in (Figure and Table 4.) The results of varieties showed that maximum roots were observed (7.75 and 9.95) were recorded in Sarsabz and Khirman. The results of concentrations indicated that maximum roots were obtained (13.60) under concentration of RTI-2, followed by (11.00) under concentration of RTI-3 and minimum roots (3.40) was achieved under concentration of MS (control) + sugar 30 g /Lunder control. The results of varieties and concentrations interactions indicted that maximum number of roots (12.60 and 14.60) under concentration of RTI-2, followed by (10.20 and 11.80) were obtained under concentration of RTI-3 and minimum roots was achieved (2.80 and 4.00) under MS (control) + sugar 30 g /L in Sarsabz and Khirman respectively. The results agreed with [13, 14].

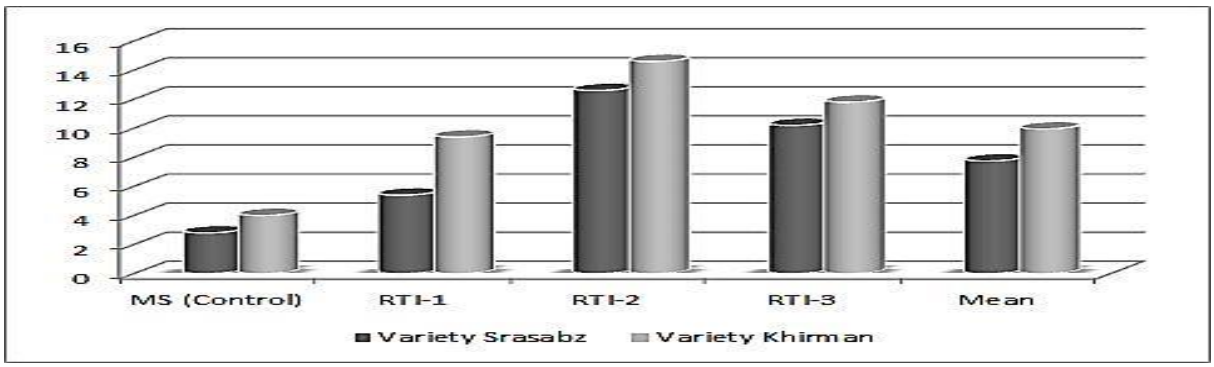


**Figure 4. Root induction in wheat varieties**

**Table 4.Root induction (g) supplemented with different concentrations of growth regulators**

| Concentrations | Varieties | | Mean |
|---|---|---|---|
| | Sarsabz | Khirman | |
| MS (Control) + sugar 30 g /L | 2.80 e | 4.00 de | 3.40 d |
| RTI-1 | 5.40 d | 9.40 c | 7.40 c |
| RTI-2 | 12.60 b | 14.60 a | 13.60 a |
| RTI-3 | 10.20 c | 11.80 b | 11.00 b |
| Mean | 7.75 b | 9.95 a | |

Analysis of Variance

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Replications | 4 | 2.600 | 0.6500 | | |
| Varieties | 4 | 8.820 | 8.8200 | 11.76 | 0.0015 |
| Treatments | 4 | 89.800 | 22.4500 | 29.93 | 0.0000 |
| V x T | 4 | 0.280 | 0.0700 | 0.09 | 0.9840 |
| Error | 36 | 27.000 | 0.7500 | | |
| Total | 49 | 128.500 | | | |

## Conclusion

It was concluded that good proliferation and callus were observed with MS + 2 mg /L 2, 4-D as compared to Picloram and NAA. The results showed that best medium MS + IAA 2.5 mg /L, IBA 2.5 mg /L + 2.5 mg /L BAP + 25 g /L for regeneration, while MS + IBA 2.0 mg /L + sugar 20 g /L for root induction under different concentrations growth regulators.

## Authors' contributions

Conceived and designed the experiments: M Naz, Performed the experiments: M Naz, Analyzed the data: M Naz, F Nizamani, SU Rehman & S Ahmeed, Contributed reagents/materials/analysis tools: M Naz, MR Nizamani & S Ahmeed, Wrote the paper: M Naz, MR Nizamani & N Ahmed.

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