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# Influence of Genotypes and Culture Media on Embryogenic Callus Introduction from Immature Embryos of Wheat (*Triticum aestivum L.)*

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Research Article

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## ABSTRACT

The contemporaneous research was directed in experimental laboratory of Department of plant Breeding and Genetics, Sargodha Pakistan to develop Invitro plant redevelopment from immature embryos of wheat, eight wheat genotypes during February 2017 to september2018 with an assessment to study the effect of genotypic of 5 Pakistani spring wheat varieties and 3 chines winter wheat varieties on callus development from immature wheat embryos. MS basal salts with 1,2,3, mgL-1 2,4-D and control was examined for callus data was recorded for different parameters. Finally, it is concluded that among the 8 spring and winter wheat genotypes Ujalla-2016 and Galaxy-2013 responded best in callusing and days taken to callusing at 1 mg/l 2, 4 –D whereas Saher-2006 performed better in callus initiation percentage. Therefore, these verities may be used to create invitro somatic variation efficiency through callogenesis.

## INTRODUCTION

Wheat (*Triticum aestivum L*) is important member to family Poaceae. Due to staple and chief nutrients grain wheat has leading position in cereals of the Pakistan. Since 1960s wheat is the supreme important nutrients of the all God's creatures and it fulfills the daily needs of people. In Pakistan it has prominent rank in cropping system. It covers about 9.170 million hectares and assembly of 25.588 million tones (Economic survey of Pakistan). With the increase in the human population of Pakistan it is assessed that it is necessary to increase the production of wheat up to 40% in 2020 to meet growing mandate of human population. Main factors which effect the wheat yield and growth are temperature, drought, disease, moisture, soil. Wheat yield has been increased since green revelation through conventional breeding but it has many constrains because they are slow and laborious.

Wheat researchers are everlasting searching for proficient methods of developing cultivars with improved yield.

The major objective of wheat breeding is to enhance the grain yield of the crop plant. For genetic variations both conventional and modern techniques are used. Among modern methods tissue culture procedure is correspondingly used to generate genetic erraticism in wheat for crop perfection. Plant tissue culture major breakthrough in improving the agronomic characters of crop plant. Main explant that was used for callus initiation and callus regeneration of wheat are immature and mature. In tissue culture protocol both undeveloped and developed emberyos are widely used [1]. But genetic transformation only undeveloped wheat embryo is the chief source anonymous. To improve the wheat different plant tissue culture technique were used by different researchers. To create hereditary variability in wheat somatic cell culture is a good source. It is besides used to rise wheat germplasm and increase in amount of hereditarily reformed plant material that act in plant breeding. Genetic variation in wheat genotypes is existing for callus initiation, redevelopment callus formation, reaction to sub-culture and plant redevelopment ability. For plant regeneration callogenesis of Immature embryos of wheat are more stable and profitable as relate to mature embryos. It was observed that in the wheat ability of totipotency is very low because the entire cell does not express this phenomenon.

Lately numerous methods are usual for set up of *invitro* cultures articulating totipotency. Establishing of procedure for callogenesis and organogenesis is compulsory for the fruitful presentation of recent biotechnology in plant perfection especially in wheat through alteration processes. Described that different parts of wheat plant were used as explant and then immature embryo were the best source for plant callus a rapidly proliferated and identical mass of cell initiation and regeneration. Based on the review of previous scientific research work on callus initiation from immature wheat embryos, the current study was planned to develop callogenesis lab Protocol by using exotic and local high yielding wheat varieties with objective to create somatic variation in wheat through immature embryos.

## MATERIALS AND METHODS

The existing investigation was directed in experimental laboratory of Department of plant Breeding and Genetics, University of Sargodha Pakistan to develop *Invitro* plant regeneration from wheat immature embryos of eight wheat genotypes.

### Source material

Three Chinese winter wheat genotype viz: C-10, C-19, C-21 and five Pakistani spring wheat varieties viz Ujalla 2016, Galaxy 2013, Punjab 2011, Faisalabad 2008 and Saher 2006 were used. In this experiment Seed of eight wheat genotypes (spring and winter wheat) were collected from the ongoing wheat experiment in the experimental area of Department of Plant Breeding and Genetics which was sown in 2016-2017.

### Source of explant

Immature embryos were used as explant basis.

### Callus initiation media

The culture medium contained MS media was used for Callus Initiation.
Recipes:

| | |
|---|---|
| M.S Media | 4.42 g /l |
| Sucrose (Sugar) | 30 g/l |
| Gel rite | 1.8 g/l |
| 2, 4 D | Varied in each Treatment (0 to 3 mg/l) |

Media was prepared different concentration of 2 4-dicholorophenoxyacetic acid (0, 1, 2 and 3) by keeping pH range from 5.5 to 5.7. Pyrex test tube of 2 mm in width and 150 mm in length were used for culture media. About 3/4 of each test tube was filled with media and air tight with polypropylene sheet and rubber band. Media was autoclaved at 1210C for 15 mints maintaining psi. Media was placed in laminar flow hood after autoclave to cool down and for solidification. Pointed forceps and scalpel were also autoclaved. 2 4-dicholorophenoxyacetic acid application for Callus initiation from immature wheat embryo [2].

T0-MS+0 mg / L 2 4-dicholorophenoxyacetic acid (Control)
T1-MS+1 mg /L 2 4-dicholorophenoxyacetic acid
T2-MS+2 mg /L 2 4-dicholorophenoxyacetic acid
T3-MS+3 mg /L 2 4-dicholorophenoxyacetic acid.

### Immature embryo culture

Immature embryos were composed from the field at milky stage 13-17 days after fertilization and these were stored at 40C in Refrigerator. Collected healthy immature Seed were separated from spikelets gently and put in a sterilized glass beaker. Surface sterilization was done by 70% ethanol for 3 minutes. Seeds were washed with d2$H_2O$ for 5 minutes. After this immature seed were pour into 10 percent sodium hypo chloride(v/v) and tween 20 solution (v/v) for disinfection three washing were done with double distil water.

### Immature

Seeds of eight genotypes were placed in laminar flow hood. Immature embryos of all genotypes were removed with the help of surgical blade and by using stereoscope in laminar flow hood. One embryo per test tube was planted. Keeping in view that scuttlum side should be up side in such a way that three fourth should be placed in solid nutrient media. Cultured test tubes were placed vertically and transferred into growth room temperature 25 ± 20C. Three to five weeks permitted adequate for callus initiation. The experiment was accompanied by adopting Completely Randomized Design (CRD) in 2 factorials with three treatments laterally with control [3]. Individually treatment repeated thrice with ten test tubes per genotype per repeat. Statistical analysis was performed by using R-statistically envirment. Analysis of Variance (ANOVA) was calculated and means were related through least significant differences.

### Data collection and growth measurement

Data of following characters was recorded after 5 weeks' inoculation of immature wheat embryos. The frequencies of callus initiation and regeneration were noted permitting to subsequent procedures after twenty and forty-five days of culture correspondingly.

## RESULTS AND DISCUSSION

### Callus initiation frequency

Statistically analysis regarding callus initiation indicated that all genotypes showed significant differences among them at all concentration of 2 4-dicholorophenoxyacetic acid. Wheat immature embryos from all studied wheat genotypes but the frequency of callus initiation was varied from genotype to genotype at altered levels of 2 4-dicholorophenoxyacetic acid concentration. Our results were in contract it was observed that all studied wheat genotypes revealed significant difference among them-selves and frequency of callus initiation varied from genotype to genotype at diverse intensities of 2 4-dicholorophenoxyacetic acid concentration in MS media. The immature embryos of chines winter wheat genotype displayed supreme callus initiation frequency (85%) at 3 mg/l of 2 4-dicholorophenoxyacetic acid followed by C-19 and Faisalabad -2008 which exhibit 75% callus initiation frequency at same concentration. Saher-2006 reveled contrast result where callus initiation frequencies were 80%, 70 % and 35% at 1, 2 and 3 mg/l 2 4-dicholorophenoxyacetic acid, respectively. Our finding is in agreement with described that the MS medium accompanied with high dose of 2 4-dicholorophenoxyacetic acid displayed the best comeback for callus initiation from immature wheat embryos. Reported maximum 88.5%-100% callus initiation percentage from immature wheat embryos. The MS without 2 4-dicholorophenoxyacetic acid (control) did not show any callus initiation in all studied genotypes. Callus stimulation and regeneration in this research have demonstrated to be genotype–reliant on and strongly subjective by the constituents of the medium used. Other reports are also in relation with our outcomes [4].

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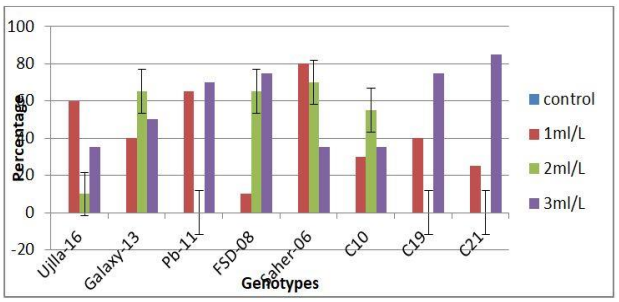
**Table 1.** Initiation frequency at same concentration.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| R | 2 | 245.3 | 122.67 | 6.48 | 0 |
| V | 7 | 3992.7 | 570.39 | 15.84 | 0 |
| T | 2 | 2789.2 | 1394.58 | 9.71 | 0 |
| V×T | 14 | 11973.8 | 855.27 | | |
| Error | 46 | 4050.5 | 88.05 | | |
| Total | 71 | | | | |

| Genotype | Mean | Homogeneous Group |
|---|---|---|
| V3 | 67.778 | A |
| V7 | 59.444 | AB |
| V2 | 56.111 | BC |
| V5 | 55.833 | BC |
| V4 | 50.556 | BCD |
| V8 | 47.222 | CD |
| V6 | 45.833 | D |
| V1 | 43.333 | D |

It was observed that over all calli initiation started three to four days after inoculation of wheat immature embryos from all studied wheat genotypes but the frequency of callus initiation was varied from genotype to genotype at diverse intensities of 2 4-dicholorophenoxyacetic acid Concentration. The immature embryos of chines winter wheat genotype exhibited extreme callus initiation frequency (85%) at 3 mg/l of 2 4-dicholorophenoxyacetic acid followed by C-19 and Faisalabad -2008 which exhibit 75% callus initiation frequency at same concentration. Saher-2006 reveled contrast result where callus initiation frequency was 80%, 70 % and 35% at 1mg/l, 2 mg/l and 3 mg/l of 2, 4 –D concentration, respectively. Control (0 mg/l) treatment displayed no callus initiation in all studied genotypes (Figure 1).

**Figure 1.** Callus initiation percentage from immature wheat embryos on different concentrations of 2 4-dicholorophenoxyacetic acid.



## Days taken to callus initiation

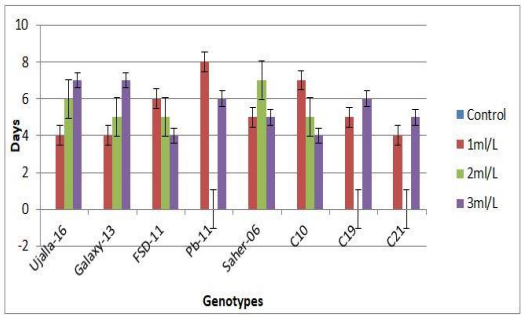
The Analysis of Variance (ANOVA) of days taken to callus initiation from immature wheat embryos no difference among all studied wheat genotypes at concentration 1, 2,3 mg/l of 2, 4 –D. LSD compression also showed that all wheat genotypes were similar for days taken to callus generation at different concentration of 2 4-dicholorophenoxyacetic acid. however the callus initiation on concentration of 2 4-dicholorophenoxyacetic acid indicate that Galaxy-2013 and C-21 took minimum days for callus initiation at concentration 1 mg/l of 2,4 –D while Punjab-2011 took maximum days (8 days) for callus initiation at same dose of 2, 4-D. The present study in agreement minimum days (4 days) for callus initiation when MS media was accompanied with 3 mg/l of 2 4-dicholorophenoxyacetic acid [5]. Defined that the MS medium accompanied with 6.0 mg/L of 2 4-dicholorophenoxyacetic acid displayed the chief comeback for callus initiation from immature wheat embryos. Investigated that the mature embryos ineffectual to initiate any kind of calli at low concentrations of 2 4-dicholorophenoxyacetic acid resultant only in primary amplification. Described that rise of 2 4-dicholorophenoxyacetic acid concentration in culture media created good callus from immature embryo of wheat. In the current experiment, it was detected that supreme number of small size callus were formed in high concentration of 2 4-dicholorophenoxyacetic acid but took more time for callus initiation. The outcome of the present experiment shown that low dosage (1 mg/l) of 2 4-dicholorophenoxyacetic acid in MS media was good to flinch callus initiation in minimum days from immature wheat embryos.

**Table 2**. Days taken to callus initiation.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| R | 2 | 7.1523 | 3.57616 | 0.66 | 0.7007 |
| V | 7 | 4.4583 | 0.6369 | 0.5 | 0.6087 |
| T | 2 | 0.9622 | 0.48111 | 1.23 | 0.2845 |
| V×T | 14 | 16.569 | 1.1835 | | |
| Error | 46 | 44.0977 | 0.95865 | | |
| Total | 71 | | | | |

| Genotype | Mean | Homogeneous Group |
|---|---|---|
| V5 | 5.25 | A |
| V6 | 5.1667 | A |
| V4 | 5.1111 | A |
| V2 | 4.8889 | A |
| V3 | 4.8889 | A |
| V8 | 4.7778 | A |
| V7 | 4.4444 | A |

**Figure 2.** Mean percentage of Days taken to Callus initiation from immature wheat embryos on different concentrations of 2 4-dicholorophenoxyacetic acid.




## Callus Morphology

Immature wheat embryos produced both Embryogenic and non-Embryogenic calli. Embryogenic calli were proliferated and compact, with a white to pale yellow apparent, whereas non-embryogenic calli were soft, watery, and shining. Greenish cream proliferated calli with excellent quality for orgenesis were observed in Galaxy-2013, Ujall-2016, Saher-2006 and C-19 at concentration 1 mg/l of 2, 4 –D while Punjab-2011 and Faisalabad -2008 show excellent result at concentration 3 mg/l of 2 4-dicholorophenoxyacetic acid. The immature embryos of C-10 produced greenish -creamy with proliferated and high quality Callus at 2 mg/l 2 4-dicholorophenoxyacetic acid. In agreement with that informed. The lowest or no callus were recorded at control (M.S.+0 mg/L of 2, 4 –D) formed no callus for all genotypes under studied.

## CONCLUSION

Finally, it is concluded that among the 8 spring and winter wheat genotypes Ujalla-2016 and Galaxy-2013 responded best in callusing and days taken to callusing at 1 mg/l 2, 4–D whereas Saher-2006 performed better in callus initiation percentage. Therefore, these verities may be used to create *invitro* somatic variation efficiency through callogenesis.

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