

Original Article

A Comparative Analysis Between Two Varieties of Wheat by Means of *in-vitro* Propagation

Ameer Hamza Baig¹, Ammarah Hasnain^{1*} and Shazia Kanwal²

¹Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan

²Department of Plant Biotechnology, Lahore College for Women University, Lahore, Pakistan

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ABSTRACT

With the increasing population, the world is facing a decrease in food production and an increase in hunger. Scientists and researchers are now trying to develop techniques to isolate the high yield varieties of staple food crops and enhance the cropping structure. In this study, two varieties of wheat (*T. aestivum L.*) are selected, and made a comparative analysis of them is carried out by making callus culture. Callus culture will give the idea about the growing capabilities of each variety. **Methods:** In vitro propagation technique is used to induce callogenesis with the help of PGR. Auxin is used as a PGR, whereas embryos were explants used for the experiment. The experiment is divided into these stages: Surface sterilization, Stock solution formation, Media formation, Embryo excision, and Frequency of Callogenesis (%). **Results:** Out of 60 excised embryos 18 and 12 calli were formed for Galaxy-2013-2013 and Faisalabad-2008-2008 respectively. Calli of Galaxy-2013-2013 was visible after 8 days and calli of Faisalabad-2008 were visible after 14 days. The frequency of callogenesis of both varieties was found to be 30 and 20% respectively. **Conclusion:** Both varieties of wheat can be cultivated on large scale and are able to generate greater yields as compared to other wheat varieties.

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Corresponding author:

Ammarah Hasnain
Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan
Ammarahhasnain3@gmail.com

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INTRODUCTION

Our world is passing through hunger crisis. A survey showed that there were about 820 million people who slept hungry every night and there will be more 260 million who will enter this list due to covid-19 economic crisis [1]. Scientists and researchers are now trying to overcome these hunger problems by increasing the food production and food availability to the people. Tissue culturing technique is being practiced to overcome the constraints that are faced during conventional methods of plant propagation [2]. Like many countries Pakistan is also suffering from different crisis including food due to over population [3]. Among many crops' wheat is the most cultivated crop of Pakistan and the 3rd most important crop around the globe after rice and maize [4]. Wheat is the essential diet of population as it constitutes 60% of the daily diet of common man in Pakistan. The average per capita consumption of wheat is about 140 kg and it occupies a central position in agricultural policies of the government of Pakistan [5]. Among many different varieties of wheat grown in Pakistan, there are two strains Galaxy-2013-2013 and Faisalabad-2008-2008. These two varieties are the specimen of our experiment. Faisalabad -2008 resembles the area of Pakistan where it grows. While the Galaxy-2013-2013 is a hybrid specie. Galaxy-2013-2013 is developed from a three-Waycross-through hybridization between Punjab-96, Watton, and MH-97.[6]. In vitro propagation is also called micropropagation because the source or the explants we use is very small can be at the micro-level. These explants can be seeds [7]; can be leaves [8], cotyledon [9], and embryos [10]. All the genetically modified plants were cultivated on trial bases first In vitro in labs and then transferred to the soil in they survive the trial. Hybrid plants were also being produced by my technique of In vitro propagation. Isolation of disease-free plants is also an application of in vitro propagation [11]. Callus culture is also an

application of in vitro propagation activity of different metabolites and making plants stress resistance is now possible through in vitro callus culture. Early plant developmental stages have been studied and are now only possible through In vitro culture. [12, 13] According to the current population scenario and the crisis related to the agriculture process, there will be massive shortage of food expected in near future. [14] Scientist and researchers are trying to find out a way to increase the efficiency of our farm lands to evaluate the efficiency of our crops and to isolate the species of our daily crops which gives the best yield. In order to contribute in the struggle to find more efficient specie, we arranged an experiment which gives comparative analysis to two strains of *T. aestivum*: one is Galaxy-2013 and the second is Faisalabad 2008.

METHODS

In this experiment Callogenesis is induced by the means of *In vitro* propagation with help of a PGR. Auxin is used as a PGR that will induce Callogenesis to the explants. The auxin especially the 2, 4-Dichlorophenoxyacetic acid (2, 4-d) has ability to induce Callogenesis to explants. The explants used to perform the experiment are the embryos excised from the wheat seeds. The results are based on the ratio of callus produced over embryo excised. We divided the experiment into 4 experimental phases: Surface sterilization, Stock solution formation, Media formation, Embryo excision, Frequency of Callogenesis (%). Surface sterilization or in this case seeds surface sterilization. Technique based on series of process to make clean and sterilized seeds. This technique follows some protocols and few ingredients to proceed the phase of seed sterilization [15]. For callus induction medium, PGR auxin was used. The auxin has the ability to increase callus expansion. Before adding in the culturing medium first we need to make stock of PGR. For that purpose, required equipment and material was used.

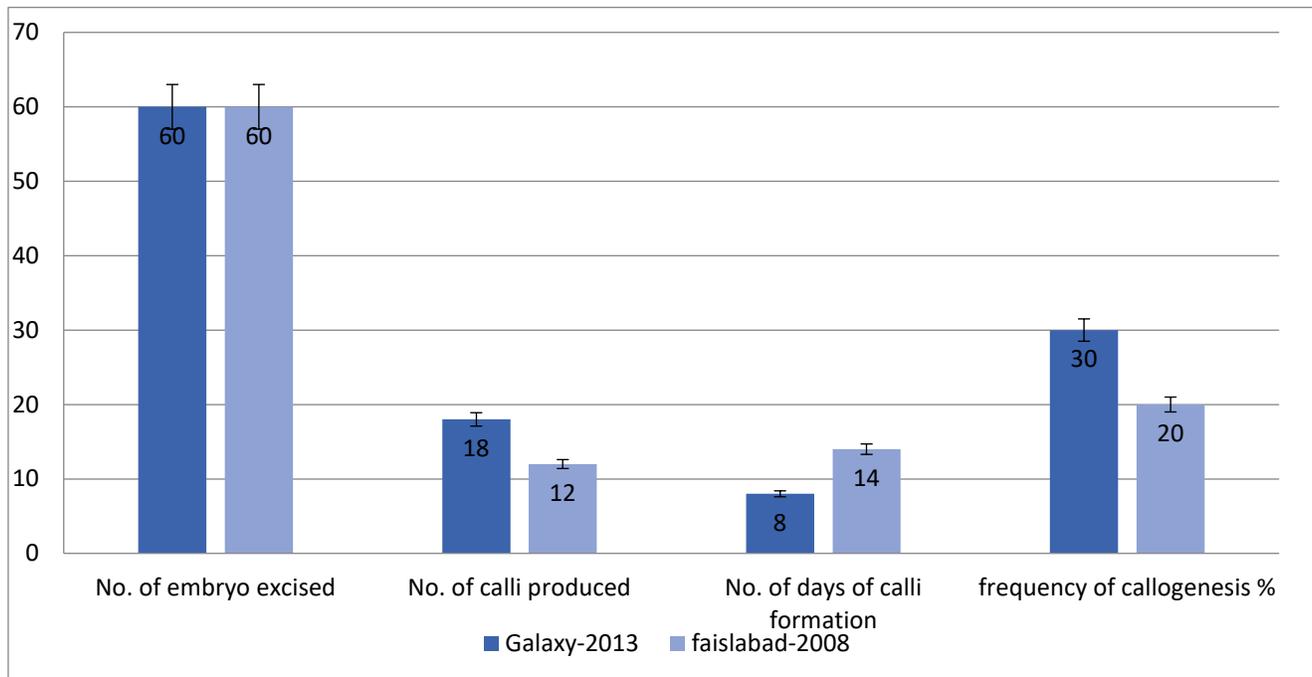
Volume in ml	NaOH in grams	Molarity
100ml	4grams	1M
500ml	19.95grams	1M
1000ml	40.00 grams	1M

Table 1: 1M NaOH solution formation

Now we have 1M solution of NaOH. For making 3mg/ml stock of 2,4-Dichlorophenoxyacetic acid add 1 ml or NaOH to falcon and add 2,4-Dichlorophenoxyacetic acid according to the required quantity.

Quantity	Volume of ddH ₂ O
3mg	1ml
30mg	10ml
60mg	20ml

Table 2: 2,4-Dichlorophenoxyacetic acid stock preparation



By using this proportion, we created stock of 2, 4-Dichlorophenoxyacetic acid. Make sure all the material is completely sanitized. 2, 4-Dichlorophenoxyacetic acid stock must be stored under -20 degrees Celsius. **Media formation:** For the experiment Murashige and Skoog (MS) media was selected. This media has all the properties of a good tissue culture media and to induce Callogenesis in the explants, we added 2, 4-Dichlorophenoxyacetic acid. Now after this, the prepared media is called callus induction media or M1 (3mg/1000ml of 2, 4-Dichlorophenoxyacetic acid). **Embryo excision:** Embryo excision is a very critical step with resemblance to callus production. The procedure of embryo excision is performed by using specific apparatus used to operate the seeds. **Frequency of Callogenesis:** Frequency of callogenesis (%) is determined by following formula:

$$\text{Frequency of callogenesis \%} = \frac{\text{number of calli produced}}{\text{number of embryo excised}} \times 100$$

RESULTS

Figure 1: Graphical representation of the performance of Galaxy-2013-2013 and FSD-2008 for calli formation, number of days of calli formation, frequency of Callogenesis (%).

Figure 1: Graphical representation of the performance of Galaxy-2013-2013 and FSD-2008

Figure 1: Two wheat varieties (Galaxy-2013-2013 and Faisalabad-2008-2008) were used in the current study. For each variety, 60 embryos were excised and placed over callus induction medium. Out of 60 excised embryos 18 and 12 calli were formed for Galaxy-2013-2013 and Faisalabad-2008-2008 respectively. Calli of Galaxy-2013-2013 was visible after 8 days and calli of Faisalabad-2008 were visible after 14 days.

Frequency of Callogenesis:

Frequencies of Callogenesis of both varieties of wheat are found by the following formula.

$$\text{Frequency of callogenesis (\%)} = \frac{\text{number of calli produced}}{\text{number of embryo excised}} \times 100$$

Frequency of Callogenesis of Galaxy-2013-2013

$$\begin{aligned}\text{Frequency of callogenesis \%} &= \frac{18}{60} \times 100 \\ &= 30\%\end{aligned}$$

Frequency of Callogenesis of Faisalabad-2008

$$\begin{aligned}\text{Frequency of callogenesis \%} &= \frac{12}{60} \times 100 \\ &= 20\%\end{aligned}$$

DISCUSSION

The present research work revealed that callogenesis is achievable by using selected cultivars and specific media conditions. Both the varieties of wheat Galaxy-2013 and Faisalabad-2008 show callus formation if protocols were followed accurately. Wheat produces calli from these explants in this case the explants were embryos excised from the seeds of wheat varieties under consideration. Different studies carried out by Nalawade *et al* in 2004 and Page Sr *et al* in 2020 reveal that wheat callogenesis is achievable via in vitro propagation technique [16,17]. Callus induction media is basically the Murashige and Skoog media which provides nutritional support and it is been proven that the MS media is best for micro propagation techniques. MS basal media is used in this experiment mixed with additional sucrose and glucose to provide sugar as energy source for the embryos. MS media is further inoculated with 3mg/ml stock of 2, 4-d to induce callogenesis in our cultural media. This auxin has been proved to be a natural hormone for callus initiator by two studies carried out by Akinyosoye ST *et al* 2014 and Lianos T in 2018 [18,19]. The results obtained from the culturing embryo clearly show that the cultivar of wheat Galaxy-2013 performed better compared to wheat cultivar Faisalabad-2008. The variety Galaxy-2013 shows it producing calli after 8 days on the other hand the varieties of wheat Faisalabad-2008 calli are observable after 14 days passing. The quantitative edge the wheat cultivar Galaxy-2013 having over the cultivar Faisalabad-2008 is not only helpful to check regeneration properties but also in cause of observing genetic changes like soma clonal variation. Higher callogenesis frequency and immediate calli growth makes the wheat variety Galaxy-2013 more favorable over Faisalabad-2008 [20].

CONCLUSIONS

The final study concluded that Callogenesis is achievable by using these wheat cultivars galaxy-2013 and Faisalabad-2008 under specific media conditions. Both of these cultivars show significant results by producing calli in callus induction media. The variety of wheat Galaxy-2013-2013 shows higher frequency of Callogenesis over the Faisalabad-2003 under less period of time.

Wheat variety Galaxy-2013 has a better callogenesis frequency in comparatively less time compared to variety Faisalabad-2008. Therefore, Galaxy 2013 can be selected for performing further experimentation and field trials.

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