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Original Article

Effect of Different Media Types on *In Vitro* Wheat Germination

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ABSTRACT

Wheat is a very crucial cereal for us as we highly depend on it and in Pakistan it is a crop which is mostly consumed. Wheat prevents colon cancer as it is a good source of dietary fiber. In vitro plant tissue culture is an alternative way to produce high yielding crops. Therefore, through the in vitro propagation of the wheat we can produce wheat with better quality and high nutritional value. **Objective:** To determine the rate of germination of the seeds of a wheat cultivar on different types of nutrient media. **Methods:** In the current study, two different types of media containing different hormones were used to investigate their effect on seed germination. For this purpose, M1 media with 2,4-D and M2 media with a combination of 2,4-D and BAP was used. Plant growth regulators were added in order to induce the germination process. The wheat cultivar Galaxy-2013 was used where 60 seeds were used for each medium. **Results:** The germination frequency on M1 was 41.66% while the germination frequency on M2 media was 61.66%. **Conclusions:** Hence, the results indicate that the combination of the PGRs showed better germination on MS media. This experiment could help in the selection of media which have a better germination capability for future research.

INTRODUCTION

Wheat (*Triticum aestivum L*) is a staple food which is widely and most commonly used as a cereal grain, it belongs to the family of the poaceae. Wheat is one of the few crops which is grown globally. The area devoted to wheat cultivation is 220.4 million hectares [1]. Due to its gluten protein, nutritional value, viscos elastic properties wheat is considered to be the most essential crop and due to its increasing demand, it is aimed to create a gene revolution in such a way that it could be made tolerant to various biotic and abiotic stresses [2]. To preserve the genetic diversity in the seed bank in vitro tissue culture is most important. Through in vitro tissue culture techniques better understanding of the metabolism and the effects of plant hormones could be understood which could eventually help in the production of the better-quality yield [3]. As compared to the conventional propagation techniques this technology has more advantages. Growth and development of the in vitro propagated plants is dependent on the genetic expression of the plant [4]. Genotype and expressions of the in vitro propagated plants are also affected by the environmental conditions. In vitro propagation can help in the production of the disease-free plantlets, decrease the period of acclimatization, fast clonal propagation, plantlets obtained through in vitro propagation have high survival rate when transferred to ex vitro conditions, reduced cost of the micropropagation plantlets etc. We can adjust the temperature, air movement, light as these physical environmental factors is predetermined in the culture medium and these factors can be varied or made constant during the growth cycle [5]. We can also adjust the chemical environmental conditions as these are also predetermined, in chemical conditions we

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can adjust the pH as well as the composition of the medium in such a way that young propagules are nourished properly [6]. The plant tissue culture room and the physical parameters in it could be optimized by changing the humidity and temperature in the room and to alter the growth conditions in a better way physically moving the culture [7]. B5, WPM and MS are the most common culture media used. Among these MS basal media is the most commonly used culture media [8]. In the MS basal media, the nitrogen content and the amount of the total salt is the highest [9]. For the growth of the explant nitrogen is the most crucial element because in the cell it affects the nucleic acid and amino acid production [10]. In this experiment MS media was used for the germination of the seeds. This study aimed to select a wheat variety i.e., Galaxy-2013 for in vitro propagation and analyzing the effect of the two PGRs i.e., 2, 4- Dichlorophenoxy acetic acid and 6- Benzyl aminopurine in MS medium on seed germination. Galaxy-2013 (*Triticum aestivum L.*) is a rust and lodging resistant, heat tolerant and high yielding variety of the wheat. The rate of germination of the seeds of a wheat cultivar was also observed by using different types of nutrient media.

METHODS

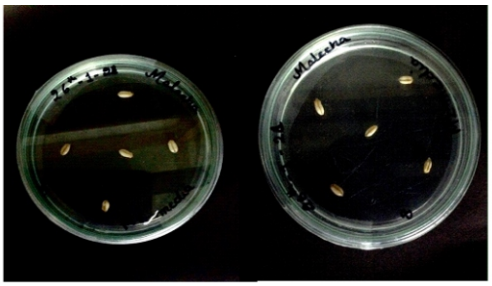
The seeds used for this experimental study were obtained from the Ayub Agricultural Research Centre and the variety of the seeds used were Galaxy-2013seeds. For the seed sterilization, the seeds were rinsed with auto calved dH20 for one minute with the half falcon filled. In the second step, ethanol 70% was used for the sterilization for about two minutes. In the third step 50% Clorox bleach was used for 15 to 20 minutes, whereas in the last step 8 to 10 times washing was done with the autoclaved dH20. Germination media (M1) was used for the seed germination purpose of the galaxy seeds which included the growth hormone 2, 4-Dichlorophenoxy acetic acid (2,4D)(3mg/L) Other nutrients in the media included were MS basal medium (1.1g), maltose (73g), Gelzan/ Gelrite (1g) for 250ml of the media. Whereas, germination media (M2) included the growth hormones 2, 4- Dichlorophenoxy acetic acid (2,4D)(3mg/L) and 6- Benzyl aminopurine (BAP) (5mg/L) [11]. Other nutrients in the M2 media included were Maltose (8.75g), MS basal media (1.1g), gelrite/gelzan (1g). These media were then autoclaved and then further subjected for the germination procedure. After the autoclave procedure, the media was allowed to be cooled down for about 10 minutes and was poured in autoclaved petri plates. For the seed placement, the seeds were first sterilized in the laminar flow with the half falcon filled with the seeds. After the last step of the washing dH20 was discarded carefully and the rest of the seeds were used for the placement. The seeds were picked with the sterilized forceps one at a time and were placed on the petri plate which contained the media in such a way that the embryonic part of the seeds was touching the media. The petri plate was then covered, wrapped with the Para film and properly labelled. For the placement of the Petri plates containing the seeds in the tissue culture lab, the rack was first properly sterilized and for the proper germination of the seeds the light above the rack was kept on whereas the other lights surrounding it were turned off. Frequency of germination was determined by following formula:

$$\text{frequency of germination\%} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds cultured}} \text{x100}$$

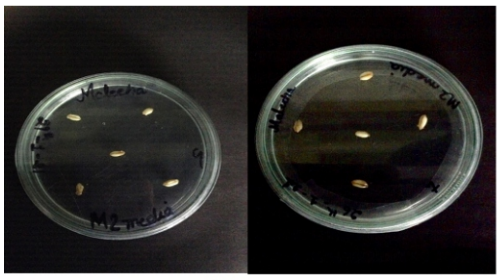
It is ration between the seeds placed and the number of seeds germinated. Multiplying the figures by 100 gives us the percentage and efficiency of our experiment.

RESULTS

Two different types of media were used to investigate their effect on seed germination of Galaxy-2013 cultivar. A total of 60 seeds were sterilized and placed on each medium. Out of 60, 25 seeds were germinated on M1 medium while 37 seeds were germinated on M2 medium (Figure 1).



(A)



(B)

Figure 1: (A) Seeds of Galaxy-2013 cultivar placed on M1 media. (B) Seeds of Galaxy-2013 cultivar placed on M2 media

Based on these observations, germination percentage was calculated. It was noticed that germination percentage was 41.66 % on M1 while it was 61.66 % on M2 media (Table 1; Figure 2)

	Total no. of seeds	No. of seeds germinated (%)	No. of days of germination
M1	60	25 (41.66%)	8
M2	60	37 (61.66%)	5

Table 1: Data on seed germination

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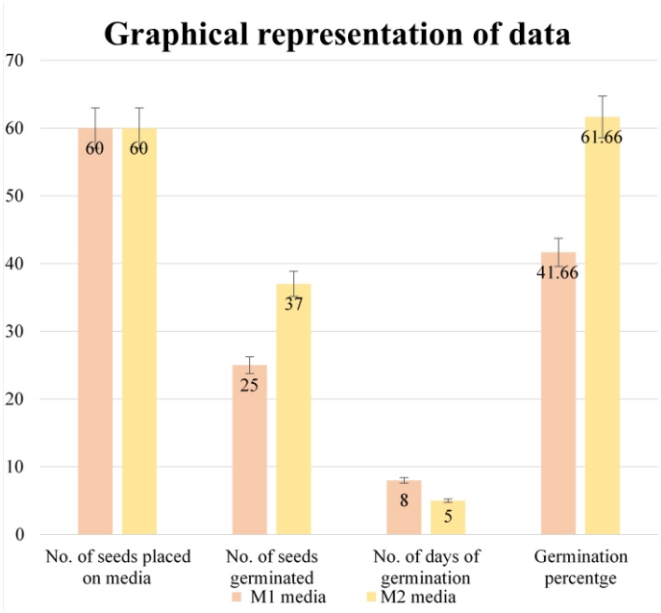

Figure 2: Graphical representation of germination data

Different stages of the seed germination were observed in M1 and M2 media as shown in Figure 3.

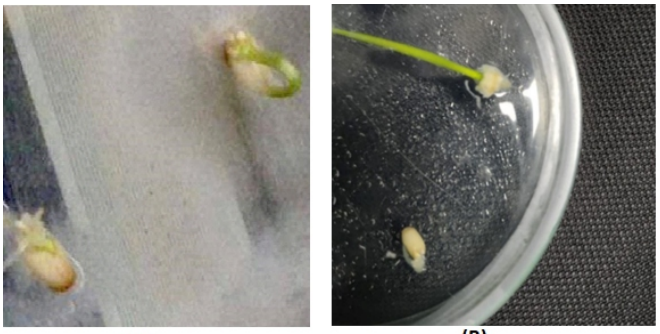

Figure 3: (A) Germination of the Galaxy-2013 seeds on M1 medium. (B) Germination of the Galaxy-2013 seeds on M1 medium

DISCUSSION

This experimental research work reveals that seed germination of wheat could be obtained by using the protocol appropriately. We used MS basal media for the germination of the Galaxy-2013 cultivar with different hormones (3mg/L of the 2,4-D and 5mg/L of the BAP). Another research work done on the germination of the (*Triticum aestivum L*) seeds used varying amounts of 2,4-D concentration which indicated that using higher than the normal concentration of the 2,4-D causes a significant decline in the seed germination [12]. A similar study indicated that the use of low dose of 2,4-D slowed down the seed germination process of wheat [13]. Therefore, in our study, a concentration of 3mg/ml of the 2,4-D was an optimal concentration for the in vitro Galaxy-2013 seed germination. In our study we studied the effect of the single PGR and combination of different PGRs on the germination of the wheat seeds. In a similar study done on wheat (*Triticum aestivum L.)* different hormonal combinations were used to observe the regeneration efficiency. In this study the BAP was used alone as well as different combinations were also observed. The RM14 media which is known as regeneration media was used which contained 2.5 mg/L of the BAP, kinetin 0.5 mg/l and IAA 0.1 mg/l. 80 to 84.5% and 83.4 to 87.9% was the regeneration efficiency obtained by it whereas 80.73% regeneration was obtained by using (0.1 mg/l) of the 2,4-D on the RM24 media. A combination of the 5 mg/l zeatin with the 2,4-D also had a good effect on shoot regeneration as well [14]. On the other hand, one of the studies observed showed that on MS medium when 0, 5 and 50 µM 2,4-D was used in combination with 0 and 0.5 µM TDZ, the shoot multiplication was slow. Whereas the root growth was also inhibited due to this combined effect. Similar results were obtained as in our study by using 2,4-D. Separately or in combination with the TDZ the shoot multiplication response was slow towards this plant growth regulator also the high concentration of the auxin: cytokinin was not so suitable for the propagation [15]. In the in vitro propagation experiments the most crucial step is the selection of a suitable media. We used MS media for the seed germination of the Galaxy cultivar. In a study conducted by Paul *et al.*, four different media have been used to study their effect on the seed germination. In this study MS medium, B5, KC and Mitra had been used and the speed of the seed germination varied according to the media which have been used [16]. After 2 weeks the greening of the embryo started to occur as compared to the other media used in which the signs of the germination started to occur later which indicates that MS media is best suited for the in vitro germination of the seeds and hence we were also able to carry out the in vitro propagation process successfully on the MS media. In our study we used 5 mg/L BAP in combination with 2,4-D in the M2 media on which the seeds of the Galaxy cultivar germinated faster followed by the rapid growth of the shoot. In many studies different concentrations of the BAP is used to assess the concentration at which the higher germination percentage occurs. Different concentrations of the BAP effect the germination of the seeds differently. A slightly different protocol followed by Gomes *et al.*, to assess the effect of the different concentrations of the BAP on the in vitro propagation of the soybean cultivar was used [17]. In our study we kept the seeds of the Galaxy cultivar placed on the media in the petri plates under the light condition whereas in this study the seeds placed on the media in the test tubes was kept under the dark. 0, 1, 3 and 5 µg / L BAP were the concentrations used followed by the different days of the pre-soaking of the seeds, this study concluded that 100% of the germination was obtained by the seeds of the

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soybean cultivar by using the 3 µg / L BAP concentration after the seven days with the pre-soaking of the seeds for 1 day. This study also concluded that increasing the concentration of the BAP followed by the increase in the days of the pre-soaking of the seeds lead to the decrease in the germination percentage. Whereas in our study we were able to obtain favourable amount (66.66 %) of the germination percentage by using 5 mg/L BAP within 5 days. In many studies the combined effect of the 2,4-D and BAP are used to investigate its effect on in vitro propagation. In a study the different concentrations of the combined effect of the 2,4-D and BAP was studied for the in vitro propagation of the saffron [18]. The concentrations used for both the hormones were (0, 0.25, 0.5, 1, 2, 4 and 8 mg L(-1)). This procedure was carried out in the darkness. This study showed that 2.0 mg L(-1) 2,4-D and 1.0 mg L(-1) BAP was the most suitable concentration for the explant [19]. In our study, we used cytokinin (BAP) along with 2,4-D which had a better germination ability. This indicates that the use of cytokinin (BAP) as a plant growth hormone accelerates the germination process. Similar results were obtained in a study, in which BAP was used for the in vitro propagation of the *Dendrobium aphyllum* (Orchidaceae) seeds [20]. The Galaxy-2013 seeds placed on M2 media containing 2,4-D (3mg/L) and BAP (5mg/L) had a better and faster germination ability as compared to the media containing only 2,4-D (3mg/L). This indicates that using a combination of the PGRs has a positive effect on the in vitro germination of the wheat seeds. Therefore, our study concluded that the concentration ranging from 3-5 mg/L is optimum for wheat germination under in vitro condition.

CONCLUSIONS

The results indicated that M2 media which contained BAP as well as 2,4-D had a better effect on the seed germination compared to M1 media. This shows that the proper amount of the cytokinin in combination with auxin should be used to achieve the better germination. Since 61.66% germination frequency was achieved by using M2 media. Hence, we can conclude that a combination of 2,4-D and BAP gives better germination. Different combinations of these auxins and cytokinin can be used in future studies.

Conflicts of Interest

The authors declare no conflict of interest.

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