

## **DEVELOPMENT OF HAPLOID PLANTS THROUGH OVARY CULTURE TECHNIQUE IN WHEAT ( *Triticum aestivum* L ).**

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### **ABSTRACT**

*This research was done at department of Plant Biotechnology, Genetic Engineering and Biotechnology Research Institute( GEBRI ), Sadat City university, Menufyia, Egypt from 2009 to 2014 years to study the effect of four factors affected callus induction of ovary cultur i.e.(cold pretreatment, chemical pretreatments, growth regulators and spikes sources. and establishment a technique to produce haploid plants. Three wheat varieties were used, Sids 1, Gemmeiza 9 and Giza 168. The results indicated that, chemical pretreatments by sterilization substances (Ethanol alcohol 70% or Mercurec chloride 1g/L or Thiourea 2g/L) were very important and essential to induce calli from ovaries. The combination between (1 and 2 mg /L 2,4-D ) and (0 , 0.1 and 0.2 mg/L kinetien) gave the highest response of callus induction and plant regeneration percentages. The estimates were increased and developed with increasing the cultured area which the spiks were collected from it. The estimates were varied with varieties.*

**Key words:** Wheat, Ovary culture, Cold & chemical pretreatments, Growth regulators, Source of spikes, Varieties.

### **INTRODUCTION**

Unpollinated ovary culture as a one of haploid production techniques has some advantages, it is an easy technique and produce more green plants than anther culture. This technique didn't take enough interest from researchers. The researchers **believe** that the cold pretreatment is very important to callus induction from megaspors, whenever they use another strongest treatment when they sterilize the spikes with chemical substances, so we must separated the cold and the chemical pretreatments, this can be applied during ovary culture process without sterilization, specially the spike is covered with the leaf sheath at the booting stage. The **frequency** of gynogenesis is low so increasing **frequency** is the ultimate goal , the highest variance was found among the spikes of different plants, so we must collect the spikes from a big cultured area. 2, 4-D was affected the cultivars

gynogenesis abilities (Madarhri–Alaoui *et al.*, 1998 and Alaoui *et al.*, 2005). Gynogenesis is **variety** dependant, many researchers were in agreement with this (Erezhenov *et al.*, 1991; Alaoui *et al.*, 2005; Salama- Ayed and Slim Amara, 2007 and Ayed *et al.*, 2010). So most researchers directed to the distant hybridization via chromosome elimination methods.

The main objective of this study was to produce haploid plants by ovary culture via determined some treatments which affected on it.

## MATERIALS AND METHODS

Seeds of the Three **Egyptian** wheat varieties, Sids 1, Gemmeiza 9 and Giza 168, which obtained from Agriculture Research Station, Gemmeiza, Tanta, Gharbeiya, Egypt were sown at plots 6 sq.m at the experimental farm of (GEBRI). Spikes were collected at the booting stage when the awn just emerged and just before the sheath **opening**, this morphological shape indicate the uninuclate stage in development of the pollen grains. leaves were removed and the stems were cut at 4 cm below the first node prior to the spike, spikes inside the sheath were washed by tap water and dried with paper towel, spikes were covered with **aluminum** foil and kept at  $3\pm 1C^0$ . Before inoculation at laminar flow spikes were immersed in Ethanol alcohol 70% at just below the first node prior to the spikes for 6 hours (except the experiment of cold pretreatment), these experiments were carried out with out sterilization, spikes were pulled from the sheath **carefully**, ovaries were excised and plated at jars each one contains 20 ml of induction medium (modified MS medium +2mg/L 2,4-D+0.1 mg /L kin +90 gm/L maltose). The ovary culture medium used in this study was based on published work in wheat (Henry and De Buyser, 1985) with some modification regarding to the kind and concentration of sugar and growth regulators. Inoculated jars were transferred to the dark at  $26 C^0$  for 4 weeks. Then jars with swelling ovaries were transferred to the light. After two weeks a creamy white callus grew out of the base of the ovary from which it was detached. Callus aged about 4 weeks were transferred to the regeneration medium (MS+0.2 mg/L. BA+200 mg /L glutamin+30 g/ L. sucrose). The regenerates were counted.

### The studied treatments :-

**1 – Cold pretreatment:** Spikes were kept at  $3\pm 1 C^0$  for ( 4, 8, 12, 16, 20, 24 and 28 **days**) before inoculation at modified MS medium+ 2mg/L 2,4-D + 0.1 mg /L kinetin +90 g/L maltose.

**2- Chemical pretreatments:** Spikes were immersed in (Ethanol alcohol 70 % or Mercurec chloride 1g/L or Thiourea 2g/L) for 3, 6, and 9 hours before inoculation at modified MS medium + 2mg /L 2,4-D + 0.1 mg/L kinetin 90 g/L maltose.

**3 – Growth regulators:** Ovaries were plated at induction medium modified MS medium with combination of ( 0 , 0.5 , 1.0 , 2.0 and 3.0 mg / L 2,4-D) and (0 , 0.1 and 0.2 mg /L kinetin) + 90 gm L maltose.

**4- Source of spikes:** Spikes of the three wheat varieties were collected from three sources:

(A) Plots, which were sown with seeds from harvesting plots of the last year.

(B) Plots which were sown with seeds from Agriculture Research Station.

(C) Open field which were sown with the same varieties.

Ovaries from the previous three sources of three wheat varieties were plated at modified MS medium + 2mg /L 2,4-D + 0.1 mg /L kinetin + 90 gm/L maltose.

## RESULTS AND DISCUSSION

### ***1-Effect of cold pretreatment period on ovary response ( callus count%) of wheat varieties .***

The results in Table 1, show the effect of cold pretreatment at  $3.0\pm 1C^0$  period on ovary response of the studied wheat varieties. All cold pretreatment periods had no effect on ovary response of the three wheat varieties, these results may be explained by, the cold pretreatment do not act as a stress as enough to switch on the genes which were responsible for the embryogenic pathway at megaspores. Unsuitable pretreatments hide the effect of medium and varieties. These results in contrast with those obtained by Sibi *et al.*, (2001) and Tsegaye Getahun *et al.* (2013).

### ***2-Effect of chemical pretreatments on ovary response (callus count % and regeneration %) of wheat varieties:***

Presented data in Table 2 clear the effect of chemical pretreatments on ovary response ( callus count % and regeneration % ) of wheat varieties

It could be noticed that ovary response differed between the studied varieties. Six **hours** was the best period to immerse the stem of the spike in the three chemicals to induce the embryogenic pathway at megaspores .Ethanol alcohol is **considered** the relatively best chemical pretreatments in comparison to the other two chemicals followed by mercurec chloride and thiourea in both estimates ( callus count and regeneration percentages). With respect to the effect of wheat varieties, Sids 1 recorded the highest callus

**Table 1:** Effect of cold pretreatment period on ovary response (callus count %) of wheat varieties.

| Cold pretreatments Period/day | Varieties |            |          |
|-------------------------------|-----------|------------|----------|
|                               | Sids 1    | Gemmeiza 9 | Giza 168 |
| 4                             | 0         | 0          | 0        |
| 8                             | 0         | 0          | 0        |
| 12                            | 0         | 0          | 0        |
| 16                            | 0         | 0          | 0        |
| 20                            | 0         | 0          | 0        |
| 24                            | 0         | 0          | 0        |
| 28                            | 0         | 0          | 0        |

**Table 2 :** Effect of chemical pretreatments on ovary response (callus count % and regeneration % ) of wheat varieties.

| Treatments |                                   | Callus count % |       |       |                          |
|------------|-----------------------------------|----------------|-------|-------|--------------------------|
| Varieties  | Chemical pretreatments            | Immersion time |       |       | Regeneration %           |
|            |                                   | ( 3 )          | ( 6 ) | ( 9 ) |                          |
| Sids 1     | C <sub>2</sub> H <sub>5</sub> OH  | 0              | 13.6  | 0     | 0                        |
|            | Hg Cl <sub>2</sub>                | 0              | 11.2  | 0     | 0                        |
|            | CH <sub>4</sub> N <sub>2</sub> S  | 0              | 10.4  | 0     | 0                        |
| 9 Gemmeiza | C <sub>2</sub> H <sub>5</sub> OH  | 0              | 8.8   | 0     | 0                        |
|            | Hg Cl <sub>2</sub>                | 0              | 6.4   | 0     | 0                        |
|            | C H <sub>4</sub> N <sub>2</sub> S | 0              | 6.4   | 0     | 0                        |
| Giza 168   | C <sub>2</sub> H <sub>5</sub> OH  | 0              | 10.4  | 0     | 100 partial regeneration |
|            | Hg Cl <sub>2</sub>                | 0              | 8.0   | 0     | 100 partial regeneration |
|            | C H <sub>4</sub> N <sub>2</sub> S | 0              | 7.2   | 0     | 0                        |



**Figure1.** Chemical pretreatment

count percentage but Gemmeiza 9 was the lowest one, Giza 168 was the only variety which exhibited partial regeneration. Every one used sterilization is considered used the previous chemical pretreatments.

**3-Effect of growth regulators concentrations on ovary response( callus count% and regeneration %) of wheat varieties:.**

The common observation of data at Table 3 is the **frequency** of ovary response of the three wheat varieties is low and the regenerates to plants do not happen, just partial regeneration only at Giza168 variety (Figure 3). These results may be due to the spikes were collected from a small cultured area ( plots which were sown with seeds from Agriculture research station) and the general frequency of ovary response is low and the highest variance was found among the spikes of different plants.

Giza 168 variety is the only variety which exhibit regeneration, this Could be due to the regeneration is higher heritability character than Callus formation .Giza 168 variety is considered the highest variety in response to ovary culture , Gemmeiza 9 variety is the lowest one meanwhile Sids 1 is the intermediate . Generally, the combination between ( 1mg /L 2,4-D , 2 mg /L 2,4-D ) and( 0 , 0.1 and 0.2 mg/L kinetin ) recorded the best results for callus count and regeneration percentages for the three wheat varieties. These results are in agreements with those obtained by ( Madarhri – Alaoui

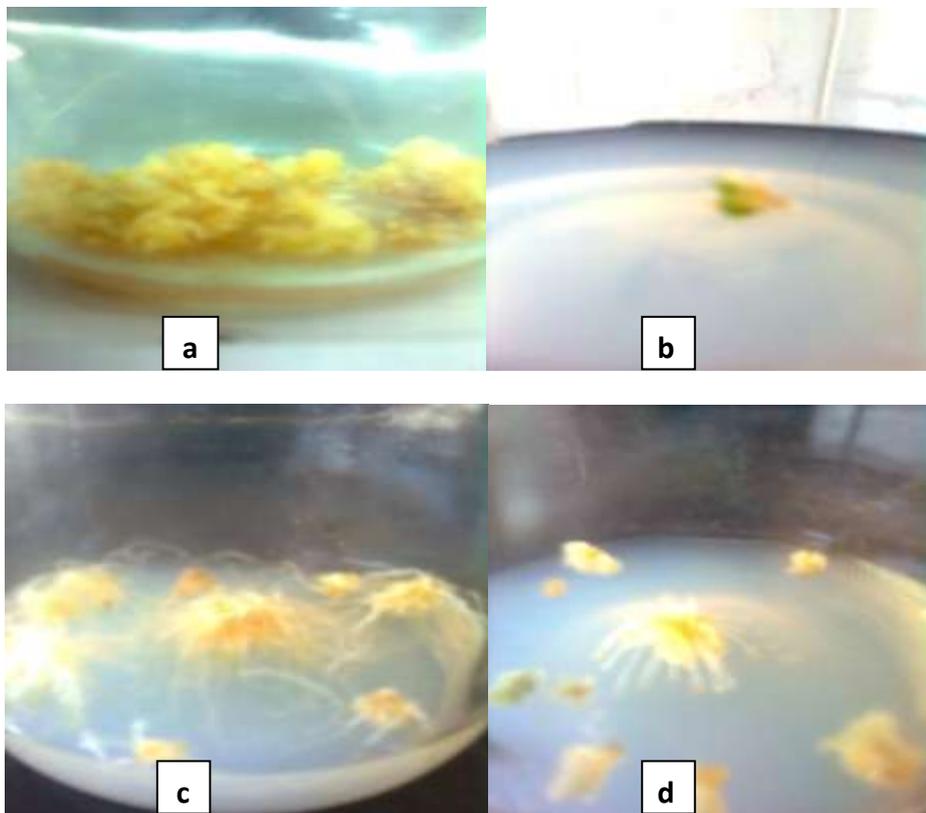
**Table 3:** Effect of growth regulators on ovary response (callus count % and regeneration%) of wheat varieties .

| Treatments |            | Varieties      |                |                |                |                |                          |
|------------|------------|----------------|----------------|----------------|----------------|----------------|--------------------------|
|            |            | Sids 1         |                | Gemmeiza 9     |                | Giza 168       |                          |
| Kin mg/L   | 2,4-D mg/L | Callus count % | Regeneration % | Callus count % | Regeneration % | Callus count % | Regeneration %           |
| 0.0        | 0.0        | 0              | 0              | 0              | 0              | 0              | 0                        |
|            | 0.5        | 0              | 0              | 0              | 0              | 0              | 0                        |
|            | 1.0        | 15.2           | 0              | 0              | 0              | 18.4           | 100 partial regeneration |
|            | 2.0        | 40             | 0              | 12             | 0              | 18.4           | 100 partial regeneration |
|            | 3.0        | 0              | 0              | 10.4           | 0              | 18.4           | 0                        |
| 0.1        | 0.0        | 0              | 0              | 0              | 0              | 0              | 0                        |
|            | 0.5        | 0              | 0              | 0              | 0              | 0              | 0                        |
|            | 1.0        | 10.4           | 0              | 0              | 0              | 0              | 0                        |
|            | 2.0        | 15.2           | 0              | 0              | 0              | 22.4           | 0                        |
|            | 3.0        | 0              | 0              | 0              | 0              | 0              | 0                        |
| 0.2        | 0.0        | 0              | 0              | 0              | 0              | 0              | 0                        |
|            | 0.5        | 5.6            | 0              | 0              | 0              | 0              | 0                        |
|            | 1.0        | 15.2           | 0              | 0              | 0              | 11.2           | 0                        |
|            | 2.0        | 16.8           | 0              | 0              | 0              | 7.2            | 0                        |
|            | 3.0        | 0              | 0              | 0              | 0              | 0              | 0                        |





**Figure 2 : Callus of ovary**



**Figure 3 : Regeneration**

*et al.*, 1998; Alaoui *et al.*, 2005 and Tsegaye Getahun *et al.* , 2013). Low concentration of 2,4-D is not enough to cell division but high concentration of 2,4-D leads to elongation of the cell without division and the toxic of 2,4-D exhibit.

**4-Effect of source of spikes on ovary response ( callus count % and regeneration % )of wheat varieties:**

Data in Table 4 show the ovary callus count and regeneration percentages as affected by wheat varieties and spike sources.

Spikes of wheat variety sids 1 which obtained from source C produced the highest percentage of callus count (47.2%) followed by spikes of the same variety which obtained from the source B (40 %). On the other hand spikes of Gemmeiza 9 variety from source A have (0.8 %) of callus count. Sids1 variety surpass the other varieties in callus count percentage meanwhile Gemmeiza 9 variety was the last.

**Table 4 :** Effect of source of spikes on ovary response( callus count% % and regeneration% ) of wheat varieties .

| Varieties  | Source of spikes | Callus count % | Regeneration %           |
|------------|------------------|----------------|--------------------------|
| Sids 1     | A                | 14.4           | 0                        |
|            | B                | 40.0           | 0                        |
|            | C                | 47.0           | 0                        |
| Gemmeiza 9 | A                | 0.8            | 0                        |
|            | B                | 12.0           | 0                        |
|            | C                | 16.8           | 0                        |
| Giza 168   | A                | 8.8            | 100 Roots                |
|            | B                | 18.4           | 100 Partial regeneration |
|            | C                | 23.2           | 0.03 Green zone          |

A : Plots which were sown with seeds from harvesting plots of the last year .

B : Plots which were sown with seeds from Agriculture Research Station .

C : Open field which were sown with the same varieties .

From the same Table 4 regeneration percentage of Giza 168 variety which obtained from the source C is **considered** the best regeneration (green zone, Figure 3b) followed by the regeneration of the same variety from the source B (partial regeneration, Figure 3a) and the regeneration from the source A (roots, Figure.3c, d) of the same variety. The regeneration exhibit only at Giza 168, this may be due to the regeneration is higher heritability character than callus formation . The regeneration to plants don,t

exhibit at the three varieties, this could be attributed to the uninucleate is unsuitable stage for ovary culture.

From these results callus count and regeneration percentages differed according to wheat variety and spikes sources while as the selection area of spikes increased callus count increased and the regeneration improved from roots to partial regeneration to green zone.

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## إنتاج نباتات أحادية باستخدام تقنية زراعة المبيض فى القمح

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أجريت أربعة تجارب خلال الفتره من ٢٠١٠ حتى ٢٠١٤ بين المزرعه التجريبيه و  
معامل قسم البيوتكنولوجيا النباتيه ، بمعهد بحوث الهندسه الوراثيه و التكنولوجيا  
الحيويه ، جامعة مدينة السادات ، محافظة المنوفيه ، مصر و ذلك لدراسة تأثير  
معاملات التبريد ، المعاملات الكيماويه بمواد التعقيم ، و منظمات النمو ، المصادر  
المختلفه للسنايل . على إنتاج النباتات الأحادية باستخدام تقنية زراعة المبيض لبعض  
أصناف القمح المصريه ( سدس ١ ، جميزه ٩ ، جيزه ١٦٨ ) فى الطور أحادى النواه  
و تأسيس تقنيه يمكن من خلالها الحصول على النباتات الأحاديه .

و يمكن تلخيص أهم النتائج كالاتى :-

- ١- لم يكن لمعاملة التبريد أى تأثير على حث المبيض لتكوين الكالس .
- ٢- و كانت المعاملات الكيماويه ضروريه جدا لحث إنتاج الكالس من المبيض أو  
حث البويضات على الإتجاه للمسار الجنينى ، يعتبر أفضلها كحول الإيثانول، يليه  
كلوريد الزئبق ثم الثيوبوريا و ذلك عند ٦ ساعات فقط من الغمس .  
و يمكن اعتبار المعامله بالمواد الكيماويه كمعاملة تعقيم وتحفيز لتكوين الكالس .
- ٣- كانت أفضل المعاملات فى استخدام منظمات النمو هى ( ١ ملليجرام / لتر 2,4-D )  
و ( ٢ ملليجرام / لتر 2,4-D ) .
- ٤- أعطت السنايل من حقل مفتوح أفضل نتائج بالنسبه لزراعة المبيض .
- ٥- التوليف بين ( ١ ، ٢ ملليجرام / لتر 2,4-D ) و ( صفر ، ١ ، ٢ ) كينتين  
سجل أعلى نتائج للكالس و التكشف .
- ٦- أعطت سنايل الصنف سدس ١ أعلى عدد للكالس بينما كان الصنف جيزه ١٦٨ هو  
الصنف الوحيد الذى سجل تكشف .

وتقترح هذه الدراسه:

- بأنه تختلف الإستجابيه لزراعة المبيض بإختلاف الأصناف حيث كان الصنف جيزه  
١٦٨ هو أفضل الأصناف فى استجابة المبيض بينما كان الصنف جميزه ٩ هو  
الأقل فى الإستجابيه بينما كان الصنف سدس ١ وسط بينهما حيث سجل أعلى كالس  
للمبيض و لكن بلا تكشف .
- تكرار تكون الكالس يعتبر منخفض بالنسبه للمبيض.