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In vitro Anther Culture of some Egyptian Rice Genotypes (*Oryza sativa* L.)

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ABSTRACT

The present research was performed at the Cell and Tissue Culture Laboratory as well as the Trial Farm of the Agronomy Department, Fac. of Agric., Al-Azhar Univ., Nasr City, Cairo. Five cultivars of rice, namely Giza 177, Giza 178, Giza 182, Sakha 102 and Sakha 104 representing a broad range of variation for several traits were utilized for this study. In the present study, four media contained MS or N6 adding with various concentrations of various growth hormones for the induction of callus from anthers. Donor plants were planted in greenhouse to study the effect of genotypes, media and their interactions. The data showed highly significant differences for callus induction, indicating the presence of genetic variation in the material used. The highest callus induction was found for Sakha 104 (18.24%) and Sakha 102 (15.16%) while , lowest callus induction was found from Giza 182 (2.33%) and Giza 178 (3.25%). The response of callus induction varied according to medium used, indicating that the M_1 medium (N6 3.99 gm/L, 2 mg/L 2,4-Dichlorophenoxyacetic acid (2,4-D), 1 mg/L Naphthalene acetic acid (NAA), 0.5 mg/L Kinetin ,3% sucrose and 8 gm/L agar) gave the highest mean value of callus induction (15.06%) , followed by M_2 medium (N6 3.99 gm/L, 2.5 mg/L 2,4-D, 0.5 mg/L Kinetin, 3% sucrose/L and 8 gm/L agar) which recorded value of callus induction (11.39%) . The interaction between cultivars and induction medium was highly significant. The studied rice cultivars were various in their response according to the medium used. The cultivar Sakha 104 followed by Giza 177 gave the highest values of callus induction on M_1 medium (28.99 and 23.33%) respectively , while the cultivar Giza 182 gave the lowest callus induction (2.00%) on M_2 or M_4 medium. Callus obtained from anther culture of rice cultivars were transferred to two plant regeneration media. Plant regeneration (%) differed among the rice cultivars. In the present study, the highest rating of green plants was found for Sakha 104 (46.00%) , Sakha 102 (33.00 %) and Giza 177 (21.00%) respectively, whereas the lowest plant regeneration produced from Giza 178 (5.00%) and Giza 182 (2.00%). It has been recorded that the cultivars that gave high callusing capacity present the best regeneration frequencies(%). Among the two plant regeneration media , the R_1 medium produced the highest green plant regeneration .These results are believed to be necessary for rice improvement utilizing the cell and tissue culture techniques. as the totipotency was necessary to the success of haploidy plants production and breeding programmes by using of cell and tissue culture techniques

INTRODUCTION

Rice is one of the most necessary crop in the world, as it provides food for more than 2 billion persons (Amornsilpa, 1998). In Egypt, rice is one of the vital cereal crops with annual growing zone of about 600,000 hectares and productivity of 5926 million tons of paddy rice. The yield about (9.88 t/ha) is believed one of the highest average yield for rice measured with the world (RRTC, 2012). Rice is one of the most necessary sources for carbohydrate for more than half of the world's population (Cassman, 1999; Khush, 2005). Anther culture is one of the biotechnological methods for the conventional plant breeding with various advantages:, rise selection efficiency, broadening of genetic difference by utilizing of gametoclonal variants, short time to be conducted by instant fixation of homozygousity and permitting early expression of recessive genes (Zapata, 1992). By the utilizing of anther culture technique, several lines as donors can be grown for various traits and these anther culture derived lines could own high yield potentiality, best grain quality, higher nutrition value , blast resistance and stem borer resistance (Draz, 2004). The conventional rice breeding methods takes 8-10 generations to obtain pure lines of a heterogeneous population. The time for the breeding process may be decreased to only 1-2 generations over the use of the anther culture system (Dewi *et al.* 1996). Anther culture biotechnology, however, is difficult to be applied on the indica rice because it is intransigent plant, which is difficult to obtain green plantlets from anther culture. Researchers in China found only about 3.0% regenerated plants of indica rice by utilizing anther culture (Zhang 1989), while the rating from crosses of indica x indica rice cultivars was lessened, about 2.0% (Zhuo *et al*. 1996).

In rice, haploids by utilizing anther culture were achieved by Niizeki and Oono (1968). The haploids could be diplodized to obtain homozygous pure lines, named double haploids, and cut short the time and cost desired to make homozygous pure lines over conventional breeding methods (Bhojwani and Dantu 2013). Anther culture, biotechnology technique, could be a supporting technique along with conventional breeding for rice improvement (De-Filippis and Ahmed, 2014). Anther culture is an easy, efficient and cost effective technique to make haploid plants in a one generation and was first written down in *Datura innoxia* (Guha and Maheshwari 1964). More than 280 varieties have been obtained with the use of doubled haploid technique in several crops (Kaushal *et al.,* 2015)

Thus, the aim of the study was to estimate the effect of genotypes, various media and their interaction on callus induction and plant regeneration on anther culture response in Egyptian rice cultivars. Also, the possibility of haploidy induction through anther culture of rice were revealed .

MATERIALS AND METHODS

The present study was performed at the Cell and Tissue Culture Laboratory and the Experimental Farm of the Agronomy Department, Fac. of Agric., Al-Azhar Univ., Nasr City, Cairo over 2015 and 2016 years. Five cultivars of rice, namely Giza 177, Giza 178, Giza 182, Sakha 102 and Sakha 104 representing a broad range of variation for several traits were utilized for this study.

Donor plants were planted in green house. Planting was done weekly interval for five sowing dates, which served as replication. The pedigree of these cultivars is present in Table (1).

Table 1. The pedigree of rice cultivars and their origin.

No.	Cultivar	Pedigree	Rice type
1	Giza 177	Giza 171 / Yomji No.1 // Pi No.4	Japonica
2	Giza 178	Giza 175 / Milyang 49	Indica/Japonica
3	Giza 182	Giza 181/ IR 39422-163-1- 2// Giza 181	Indica
4	Sakha 102	Japonica 177GZ 4096-7-1 / Giza	Japonica
5	Sakha 104	GZ 4096-8-1 / GZ 4100-9-1	Japonica

Anther culture procedure

Panicles were selected from tillers of the each cultivar in the morning (9 am to 10 am) at appropriate stage, panicles were selected when the distance between flag leaf and penultimate leaf was 5-7 cm (Croughan, 1998). Panicle was surface sterilized in ethanol 70% for 1 minute, and 5% sodium hypochlorite for 15 minutes under aseptic condition; individual spikelet was cut to obtain the anthers. Separate spikelet was taken at the base while holding from the tip, to disconnect the anther lobes from the filaments. The released anthers were placed onto agar solidified media contained in jars. Approximately 30 anthers were inoculated to each jar on medium for callus induction. Ten jars were accounted for each treatment. The jars were incubated in complete darkness at 25-28 ºC for 5-7 weeks for callus induction. The cultured plates were inspected periodically at weekly intervals to observe the progress in respect of callus induction. Data on rating of callus induction was written down. Calluses of at least 2 mm diameter were transferred from callus induction medium to regeneration media and kept under 16 h photoperiod (2000 lux) at 25-28 ºC. The cultures were inspected weekly and data on rating of regenerating green and/or albino plants was written down. In regeneration medium, some calluses lost their capacity to make plantlets and turned brown while others differentiated into green or albino plants. Green plantlets without rooting were transferred to rooting medium (half strength MS medium free hormone).

Induction medium: Anthers containing pollen at the mid or late uninucleate microspores stage of development were plated on the two induction medium used in this study were N6 medium (Chu *et al*. 1975), and MS (Murashige and Skoog (1962) containing 3% sucrose and 8 gm/l agar, 0.5 mg/L Kinetin and with two different concentrations of 2,4-Dichlorophenoxyacetic acid (2,4-D) and Naphthalene acetic acid (NAA) as the following :

M_1: N6 medium with 2 mg/L 2,4-D and 1 mg/L NAA.
M_2: N6 medium with 2.5 mg/L 2,4-D.
M_3: MS medium with 2 mg/L 2,4-D and 1 mg/L NAA.
M_4: MS medium with 2.5 mg/L 2,4-D.

Regeneration medium :

The regeneration medium was MS medium (4.43 gm/L) containing 3% sucrose and 7 gm/l agar, 1 mg / NAA and two different concentration of Kinetin as the following :

R_1: MS medium 0. 5 mg/L Kinetin.
R_2: MS medium 2. 0 mg/L Kinetin.

Visual observation of culture was recorded every week and data were taken after 4-6 weeks of inoculation. The green regenerated plants were moved to MS medium (half strength MS medium free hormone) for root formation. All the cultures were placed in growth room at a temperature of 26±2ºC. The source of light was white florescent light with strength differing from 2000-3000 lux.

Statistical Analysis

Design of experiment: The experiment was carried out in a completely randomized design with ten replicates .The data were statistically analyzed utilizing CO-STAT program version. Analysis of variance (ANOVA) was done for studying the effects of genotypes, media and their interaction. Mean comparisons were conducted utilizing the LSD test at 5% and 1% probabilities level.

RESULTS AND DISCUSSION

Importance of genotypes and nutrition media on callus induction

In this study, four media contained N6 and MS with various concentrations of growth hormones for callus induction from anthers .The anther culture determents of rice cultivars studied on four callus induction media are shown in Table (2). Results showed that there were highly significant differences for genotypes , media and their interactions, indicating the presence of genetic variation between these genotypes . It was noticed that the color of the callus induction in the experiment was dependent on genotype and the culture medium used . Callus induction differed among the rice genotypes . The highest callus induction was observed in Sakha 104 (18.24%) and Sakha 102 (15.16%). While, the lowest ratings of callus induction was observed in Giza 182 (2.33%) and Giza 178 (3.25%) (Table 2 and Fig. 1). Low rate of callus induction and plant regeneration is generally observed in anther culture with indica rice (Giza 178 and Giza 182). That the quality and frequency of callus induction and following plant regeneration could be improved by selecting better receptive rice cultivars has been suggested (Niroula and Bimb, 2009) Also, M1 and M2 gave a great number of androgenesis calluses more than M3 and M4. These results are in agreement with that obtained by Bagheri and Jelodar (2008), Niroula and Bimb (2009) and Silva (2010). Even among various cultivars of indica or japonica, much variation in pollen callusing and green plant regeneration has been showed with the genotypic effect being greater among the indica types (Silva, 2010). Results of this investigation agree with the above observations. Indica varieties had significantly low callus induction than the japonica variation.

The response of callus induction varied according to medium used, indicating that the M1 medium gave the highest mean value of callus induction (15.06%) followed by M2 medium which recorded rate of callus induction (11.39%) (Table 2).

The interaction between genotypes and induction medium was highly significant. The studied rice genotypes were various in their response according to the medium used. The genotype Sakha 104 followed by Giza 177 gave the highest values of callus induction on M1 medium (28.99 and 23.33%), respectively, while the genotype Giza 182 gave the lowest callus induction (2.00%) on M2 or M4 medium. These findings were steady with the previous reports stating the existence of significant variation in callus induction due to cultivar, media composition and their interaction (Bagheri and Jelodar 2008 and Qiumio and Zapata 1990).The results showed that callus was produced successfully from all five tested cultivars in all four media in contrast to the previous studies. GuhaMukerjee 1973 reported that only 5 out of 18 indica cultivars presented pollen callusing and callus from only one cultivar differentiated into plants. Lentini *et al* (1995) reported that only 1 out of 35 indica variation exhibited pollen callusing on N6 medium .This slight discrepancy observed in our study.On the other hand ,Sakha cultivars were higher for the *in vitro* androgenesis induction more than Giza cultivars .

Table 2. Callus induction (%)of anther culture for five rice genotypes at each induction medium.

Cultivars / Media	Giza 177	Giza 178	Giza 182	Sakha 102	Sakha 104	Means
M1	23.33	3.33	3.00	16.66	28.99	15.06
M2	11.33	4.00	2.00	20.99	18.66	11.39
M3	5.66	3.00	2.33	9.99	10.99	6.39
M4	10.33	2.66	2.00	12.99	14.33	8.46
Means	12.66	3.25	2.33	15.16	18.24	
L.S.D	**Cultivars**		**Media**		**Interactions**	
5%	**3.17**		**2.83**		**6.34**	
1%	**4.18**		**3.74**		**8.36**	

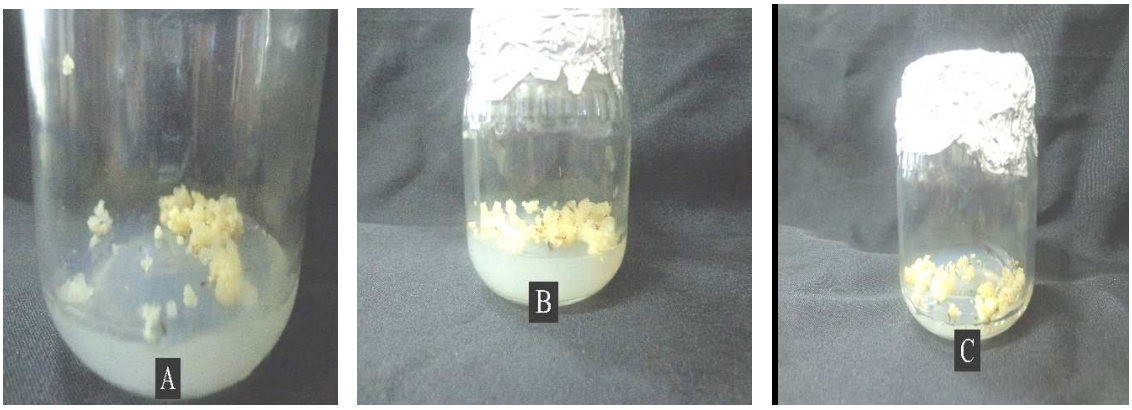

Figure 1. (A) Callus induction from rice anther culture on M_1 medium for Sakha 102 after 6 weeks from culture establishment, (B) ,callus induction from anther culture establishment , of Sakha 104 after 6 weeks and (C) callus grown after 6 weeks from culture establishment, of Giza 177 anthers

Response of various genotypes on plant regeneration media

In present study, two media contained MS was added with various concentrations of various growth hormones for plant regeneration . The induced calli from anther culture were transferred to regeneration medium and the results are presented in Table (3). Results indicated that there were highly significant differences between these genotypes, suggesting the presence of genetic variation in the material used. Plant regeneration (%) differ among the rice cultivars. The highest rating of green plants was shown for Sakha 104 (46.00%) , Sakha 102 (33.00 %) and Giza-177 (21.00%), respectively. The lowest plant regeneration was observed in Giza 178 (5.00%) and Giza 182 (2.00%) (Fig. 2). Low rate of callus induction and plant regeneration is generally observed in anther culture with indica rice (Giza 178 and Giza 182). Also, the results revealed that japonica rice cultivars (Sakha 102 ,104 and Giza 177) were regenerated to plantlets from anther callus more than the other indica cultivars at least 4-6 times .These results are agree with that obtained by Chowdhury and Mandal (2001), Javed *et al* (2007), Bagheri and Jelodar (2008) and Niroula and Bimb (2009).

(Javed *et al*. 2007 and Shahnewaz *et al*. 2003) showed that the cultivars that exhibit high callusing capacity show the best regeneration amount (%). The results obtained in our studies are constant with this trend. It was observed that quality of callus play a significant role in plant regeneration. The calluses which were milky white in color and compact in texture had the ability to regenerate plantlets. These results clearly suggest that the media has an effect on the morphogenic competence of the induction callus, determining its regeneration capability. This implies that successes of regeneration depend on callus formation for all cultivars.

Zhang (1989) observed that the rating of green plants made by anther culture from indica rice was only 3%. In cereals, the rating of green plantlets that can be regenerated is still a boundary factor in utilizing anther culture for rice breeding (Zhou, 1996). This subspecies has a high ability to anther culture response with green plantlets ranging from 20% to 60%. In general , the donor plants has inviolable roles in production of green plantlets from anther culture .These result agree with Chung (1992), Razdan (1993) and Masyhudi (1997).

Table 3. Frequency (%) of plantlets regenerated in anther cultures from various cultivars

Cultivars / Media	Giza 177	Giza 178	Giza 182	Sakha 102	Sakha 104	Means
R1	34.00	4.00	2.00	48.00	48.00	27.20
R2	8.00	6.00	2.00	18.00	44.00	15.60
Means	21.00	5.00	2.00	33.00	46.00	
L.S.D	**Cultivars**		**Media**		**Interactions**	
5%	**25.92**		**n.s**		**n.s**	
1%	**34.34**		**n.s**		**n.s**	

Figure 2. (A) start of regeneration from Giza 177 anthers on R_1 medium, (B) plantlet regenerated from Sakha 104 anthers on R_1 medium and (C) green plantlets emerging from cultured anthers measured with albino plant on R_2 medium.

Table 4. Effect of plant regeneration medium on formation of albino plants frequency

Cultivars / Media	Giza 177	Giza 178	Giza 182	Sakha 102	Sakha 104	Total
R1	2	-	-	3	2	7
R2	-	-	-	2	3	5

The rate of occurrence of albino plants (Table 4) developed was lower across the cultivars and media composition as measured to rate of occurrence of green plants. The highest albino formation was observed for Sakha 102 (5 plants), Sakha 104 (5 plants) followed by Giza 177 (2 plants). No albino formation was watched in Giza 178 and Giza 182.

Albinism is counted as a vital problem in anther culture in rice, especially in indica rice (Chen *et al*. (1991). The recovery of albino plants from pollen derived calluses has been a formidable obstacle to the utilization of rice anther culture for rice improvement (Chowdary and Mandal 2001) which might be attributable to the long culture duration and the cultivar. Literature on androgenesis in cereals suggests that albinism could be considerably decreased by shortening the culture period (i.e. frequents subculture).

Also, plants frequencies indicated the possibility of occurrence haploid plants through anther culture , this result was clearly showed from data in Table (4) which gave the albino plants from japonica rice cultivars only (Giza 177 and Sakha 102 & 104). Croughan (1998) was suggested the same results in anther culture for doubled haploid productions , also Zhou (1996) and Kaushal *et al* (2015) reported the same manner for doubled haploid anther cultures in rice.

REFERENCES

Amornsilpa, S.(1998). Hybrid rice in Thailand, En: Advances in hybrid rice technology. Manila : *IRRI*, 412 p.

Bagheri, N. and N.B. Jelodar (2008). Combining capacity and heritcapacity of callus induction and green plant regeneration in rice anther culture. Biotechnology 7(2): 287-292.

Bhojwani, S.S and P.K. Dantu (2013). *Plant Tissue Culture: An Introductory Text.* Springer Dordrecht. DOI: 10.1007/978-81-322-1026-9_1,Springer India.

Cassman, K. G. (1999). Ecological intensification of cereal production system: Yield potential, soil quality and precision agriculture. Proceeding of National Academy of Sci., 96, 5952-5959.

Chen, C.C ; H.S. Tsay and C.R. Huang (1991). Factors affecting and rogeneis in rice (*Oryza sativa* L.) in Biotechnology in agriculture and forestry, (Ed. By YPS Bajai), spiger-Verlag, Berlin Heidelberg, 14. 195-211.

Chen, Y. (1983). Anther and pollen culture of rice in China. p. 11- 26. In Cell and Tissue Culture Techniques for Cereal Crop Improvement. Proceedings of a Workshop cosponsored by the Institute of Genetics, Academia Sinica and the International Rice Research Institute. Science Press, Beijing, China.

Chowdhury, B. and A.B. Mandal, (2001). Microspore embryogenesis and fertile plantlet regeneration in a salt susceptible x salt tolerant rice hybrid. Plant Cell Tissue and Organ Culture 65:141-14.

Chu, C.C; C.C Wang; C.S Sun; C Hsu; C.Y Chu and F.Y Bi. (1975). Establishment of an efficient medium for anther culture of rice, over comparative experiments on the nitrogen sources. Science Sinica 18, pp: 659-668.

Chung, G.S. (1992). Anther culture for rice improvement in Korea. In K. Zheng and T. Murashige (Eds.). Anther Culture for Rice Breeders. Seminar and Training for Rice Anther Culture at Hangzhou, China. p. 8-37.

Croughan, T.P.(1998). Anther culture for double haploid production. In: Gamborg OL, Phillipsn GC (ed) Plant Cell Tissue and Organ Culture :Fundamental Methods Narosa Publishing, New Delhi, 143-154

De-Filippis, L. F., and P. Ahmed. (2014). *Improvement of Crops in the era of climatic change* Springer Pub., New York. 289-346. http://dx.doi.org/10.1007/978-1-4614-8830-9_12.

Dewi, I.S.; I. Hanarida and S. Rianawati (1996). Anther culture and its application for rice improvement program in Indonesia. *IARD J.*18(3): 51-56.

Draz, A. E. (2004). Contribution and utilization of anther culture lines in rice improvement in Egypt. approaches at the farm and regional levels International Rice Research Institute, Los Banos, Philippines.. 1, 263-279.

Guha-Mukerjee S. (1973). Genotypic differences in the *in vitro* formation embryoids from rice pollen. J. of Experimental Botany. 24: 139-144.

Guha, S. and Maheswari, S. C. (1964). In vitro production of embryos from anthers of Datura. Nature 204,497.

Javed, M.A.; S. Misoo ; T. Mahmood ; M.S. Haider ; A.H. Shah ; V.N. Rashid and J. Iqbal (2007). Effectiveness of alternate culture temperatures and maltose in the anther culture of salt tolerant indica rice cultivars. African Crop Sci. Conference Proceedings 8: 753-757.

Kaushal, L; S.M. Balachandran; K. Ulaganathan and V. Shenoy (2015). Assessment of first generation and rogenicrice lines for true doubled haploids. International J. of Agric. Sci. and Res., 5 (2): 41-54.

Khush, G. S. (2005). What it will take to feed 5.0 billion rice consumers in 2030. Plant Molecular Biology, 59: 1-6. http://dx.doi.org/10.1007/s11103-005-2159-5

Lentini, Z., P. Reyes, C.P. Martinez and W.M. Roca (1995). Androgenesis of intransigent rice cultivars with maltose and silver nitrate. Plant Sci., 110: 127-138.

Masyhudi, M.F. (1997). Kultur antera tanaman padi subspesies javanica. J. Penelitian dan Pengembangan Pertanian XVI (1): 30-36.

Murashige, T. P. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiologia Plantarum 15 (3): 473-497.

Niizeki, H. and K Oono (1968). Induction of haploid rice plant from anther culture. *Proc. Jap. Acad. Sc.*44 :554–557.

Niroula, R. K. and H.P. Bimb, (2009). Effect of cultivar and callus induction medium on green plant regeneration from anther of Nepalese rise cultivars. Asian J. of Plant Sci. 8(5): 368-374.

Qiumio, C.A. and F.J. Zapata, (1990). Diallel analysis of callus induction and green plant regeneration in rice anther culture. Crop Sci. 30: 188-192.

Razdan, M.K. (1993). An Introduction to Plant Tissue Culture. Oxford & IBH Publishing Co., Ltd., New Delhi.

RRTC. (2012). *Final results of 2012 growing season*. Rice Research and Training Center (National Rice Research Program), Sakha, Kafrelsheikh, Egypt.

Shahnewaz, S; M. A Bari ; N. A Siddique ; N. Khutun ; M.H. Rahman and M. H. Haque.(2003). Induction of haploid rice plants over *in vitro* anther culture. Pakistan Journal of Biological Science. 6(14): 1250-1252.

Silva, T. D. (2010). Indica rice anther culture: can the impass be surpassed? Plant Cell, Tissue and Organ Culture 100(1):1-11.

Zapata, A. F. J. (1992). Increasing anther culture efficiency in rice (*Oryza sativa* L.) utilizing anthers from ratooned plants. *Plant Sci. J., 151*, 107-114.

Zhang, Z.H. (1989). The practiccapacity of anther culture breeding in riceIn A. Mujeeb-Kazi and L.A. Stich (Eds.). Review of Advances in Plant Biotechnology, 1985-88. International Maize and Wheat Improvement Centre-International Rice Research Institute. 31-42.

Zhou, H. (1996). Genetics of green plantlet regeneration from anther culture of cereals. 169-187.

Zhuo, L.S., H.M. Si, S.H. Cheng, and Z.X. Sun. (1996). Phenyl acetic acid Stimulation of direct shoot formation in anther and somatic tissue cultures of rice (Oryza sativa L.). Plant Breed. 115: 295- 300.

زراعة المتوك من بعض التراكيب الوراثية للأرز المصري معمليا

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أجري هذا البحث في معمل زراعة الأنسجة بقسم المحاصيل – كلية الزراعة جامعة الأزهر خلال عامي 2015 و2016 وذلك بهدف دراسة تأثير إستجابة المتوك لبعض أصناف الأرز المصري (جيزة 177، جيزة 178، جيزة 182، سخا 102 و سخا 104) من حيث قدرتها علي إستحداث كالوس وإنتاج نباتات علي بيئات زراعية مختلفة حيث تم تقييمها لزراعة المتوك في المرحلة المناسبة والتي تكون فيها حبوب اللقاح غير ناضجة وتحتوي علي نواة واحدة (mid or late uninucleate microspores) وذلك علي أربعة بيئات مغذية لإستحداث الكالوس وبيئتين لإنتاج نباتات، وصممت التجربة في تصميم عشوائي كامل في عشر مكررات ، وقد سجلت البيانات علي صفتي إنتاج الكالوس وإستيلاد النباتات وقد حللت البيانات إحصائيا بإستخدام برنامج Co-stat ويمكن تلخيص النتائج المتحصل عليها فيما يلي :- - أظهر تحليل التباين وجود إختلافات عالية المعنوية بين التراكيب الوراثية ، البيئات المغذية والتفاعل بينهما علي نسبة إنتاج الكالوس ، وقد سجلت أعلي نسبة لإستحداث الكالوس للصنف سخا 104(18.24%) وسخا 102 (15.16%) بينما سجلت أقل نسبة لإستحداث الكالوس للأصناف جيزة 178(3.25%) وجيزة 182 (2.33%). وقد تأثرت نسبة إستحداث الكالوس بالبيئة المغذية المستخدمة فقد سجلت البيئة المغذية (M_1) أعلي نسبة لإستحداث الكالوس (15.06%) تلتها البيئة المغذية (M_2) حيث سجلت نسبة للكالوس (11.39%).وكان للتفاعل بين التراكيب الوراثية والبيئات تأثيرٌ معنويٌ علي نسبة إستحداث الكالوس وسجل الصنف سخا 104 متبوعا بالصنف جيزة 177 أعلي القيم لإستحداث الكالوس علي بيئة (M_1) حيث سجل نسبة للكالوس (28.99% و23.33 %) علي التوالي . - أظهر تحليل التباين وجود إختلافات عالية المعنوية بين التراكيب الوراثية علي نسبة إستيلاد النباتات ، وقد سجلت أعلي نسبة إستيلاد النباتات للصنف سخا 104(46.00%) ، سخا 102 (33.00 %) و جيزة 177(21.00 %) بينما سجلت أقل نسبة لإستيلاد النباتات للأصناف جيزة 178 (5.00 %) و جيزة 182 (2.00 %). وقد بينت النتائج أن الأصناف التي أعطت نسبة عالية من الكالوس أعطت نسبة عالية من النباتات المستولدة (%)، فيما سجلت بيئة إستيلاد النباتات (R_1) أعلي نسبة من النباتات الخضراء. ويمكن إعتبار هذه النتائج مهمة جدا في تحسين محصول الأرز بإستخدام تقنيات مزارع الأنسجة والخلايا ولا سيما بإستخدام زراعة المتوك. كما وتعتبر مصدراً هاماً لإنتاج النباتات الأحادية ذات القيمة العالية في تحسين الأصناف في النباتات أحادية الفلقة.