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ANTHER CULTURE RESPONSE OF MODERN EGYPTIAN RICE VARIETIES

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ABSTRACT: The present study aimed to investigate the response of modern Egyptian rice varieties, i.e., Sakha 101, Sakha 102, Sakha 103, Sakha 104 and Giza 177 for androgenesis and to determine the optimum hormone balance for increasing callus induction and plant regeneration abilities. The mean of callus induction frequencies in different media and media combinations ranged from 4.74 to 17.4%. The media supplemented with N6 + 1mg/l NAA + 1 mg/l 2ip ($CIMZ_2$) and B_5 + 2.5 mg/l NAA + 0.5 mg/l kn + 2.5 mg/l 2,4-D ($CIMZ_3$) were found most effective for callus induction (16.12% and 17.4 respectively). Sakha 101 and Sakha 103 possessed higher frequencies than the other varieties for response of callus induction over all the different concentrations of hormones (17.1 and 17.2). Plant regeneration frequencies from the plated callus on agrified MS medium exhibited also large variable means and ranged from 33.33 to 65.38%. The medium supplemented with MS + 0.5 mg/l NAA + 0.5 mg/l kn + 0.5mg/l 2ip ($PRMZ_2$) was found as the most effective for plant regeneration frequency (65.38). Sakha 101 possessed higher frequency than the other varieties for response of plant regenerations over all different concentrations of hormones (61.95). Sakha 102 gave lowest frequency as over all concentrations (33.33). This variety possessed higher frequency of plant regenerations (100%) under PRMZ2 medium. These results might insure the importance of interaction between this specific genotype and especial hormone blance (PRMZ2).

Highly significant differences of genotypes (A), media composition (B) and their interaction between them (A x B) were detected for no. of regenerants per callus and plantlet height, while callus diameter possessed significant (only at interaction) and insignificant occurred of genotype and media composition on this criteria. Therefore, increasing anther culture response should have extensive more trails to determine the optimum hormone balance for each genotype.

Key words : **Anther, callus, regeneration, plantlet, media, hormone.**

INTRODUCTION

Rice is one of the world's most important crops. This cereal represents the primary or secondary stable food for more than half of the global population. In Egypt about 650 thousand Feddans are cultivated in North Delta. Considerable efforts are being directed towards the improvement of rice yield. Using biotechnological techniques, the production of haploids via anther culture represents an alternative to traditional crop improvement programs. The initial success in rice anther culture by Niizeki and Oono (1968) is prompted many to use this technique in their breeding programs. The acceleration of the breeding cycle and increase of selection efficiency make double haploid techniques very attractive, not only for conventional breeding but also for plant improvement through mutation induction. Anther culture technique can be considered complementary to mutation induction because both dominant and recessive genes will be phenotypically expressed allowing easier isolation of desirable recessive mutation. Anther culture is one of the most extensively investigated areas of rice tissue culture. In China, for example, over a dozen important varieties and more than 100 improved lines have been developed through anther culture breeding (Loo and Xu, 1990). In Egypt, several rice anther culture lines are obtained (Soliman *et al.*, 1999 and Draz, 2004). Especially in rice, spontaneous double haploids (SDH) represented 46% of the regenerated green plants (Guiderdoni *et al.*, 1992). This percentage could be increased up to 65% by addition of colchicine (250- 500 mg/l) of callus induction media (Alemanno and Guiderdoni, 1994).

Among the factors affecting anther culture response in rice, a genotype and the composition of the culture medium seem to be critical for the effective induction of callus and plant regeneration from anthers (Xie *et al.*, 1995 and 1997). Different response of genotypes were reported by several investigators (Aruna and Reddy, 1988; Rout *et al.*, 1991 and Ranjan *et al.*, 1998) for callus induction and plant regeneration frequencies of anther culture. However, 2.4,D and NAA were used as auxins and kinetins and BAP as a cytokinines with different concenterations in many studies of anther culture

technique (Mandal and Gupta, 1997, Zhu *et al.*, 1998, Chandel *et al.*, 1998 and Gabriela *et al.*, 2002).

Therefore, the present study aimed to investigate the response of modern Egyptian rice varieties, i.e., Sakha 101, Sakha 102, Sakha 103, Sakha 104 and Giza 177 for androgenesis and to determine the optimum hormone balance for increasing callus induction and plant regeneration abilities.

MATERIALS AND METHODS

The present investigation was carried out at the Biotechnology, Res. Lab and Greenhouse of the Genetics Department, Faculty of Agriculture, Zagazig University. The materials of this study were consisted of five rice varieties (*Oryza sativa* L.).i.e, Giza 177, Sakha 101, Sakha 102 Sakha 103 and Sakha 104. These varieties were developed by Agricultural Research Center (ARC), Minisitry of Agriculture, Egypt. Origin and important characteristics of these varieties are illustrated in Table 1.

Seeds of each variety were sown in the summer season in greenhouse at 15th May 2000 and were transplanted after 30 days into the field. The space between each plant was 20 x 20 cm. All rice varieties were planted in randomized complete block design with three replicats. Traditional agronomic practices were applied in this study.

Panicles collection:

The exact panicles at the uninucleate stage were selected. Boots or shoots were collected when the spacing between the penultimate leaf collar and flage leaf collar is 5-9 cm., this space corresponds to the uninucleate stage. Different fresh boots were washed throughly with tap water, warpped in paper towles moistened with distilled water and subjected to cold shock at 4°C for 8 days as a pretreatment.

The procedures of anther culture technique according to (Zapata, 1985) were applied in the present investigation.

A. Media preparation:

1. Callus induction media:

Anthers were cultured on N_6 and B_5 media with four different concentrations of growth regulators as follows :

a- N_6 basic medium + 2 mg/l NAA (Naphthalene acetic acid) + 0.5 mg/l Kinetin (CIM0).

b. N_6 basic medium + 0.5 mg/l NAA + 1mg /l 2ip ($\lambda - \delta$; δ-Dimethyl allyl amino) (CIMZ1).

c. N_6 basic medium + 1mg/l NAA + 1mg/l 2ip (CIMZ2).

d. B_5 Basic medium + 2.5 mg/l NAA + 0.5 mg/l Kinetin + 2.5 mg/l 2, 4D. (Dichlorophenoxy acetic acid) (CIMZ3).

2. Plant regeneration media (Differentiation media):

Optimum size of callus were transplated into MS medium (Murashige and Skoog 1962) with four concentrations of growth regulators as follows :

a. MS + 0.5 mg /l NAA + 2 mg/l Kinetin (PRM0).

b. MS + 0.5 mg/l NAA + 1mg/l Kinetin + 1mg/l 2ip (PRMZ1).

c. MS + 0.5 mg/l NAA + 0.5 mg/l Kinetin + 0.5 mg/l 2ip (PRMZ2).

d. MS + 1 mg/l NAA + 4 mg/l kinetin (PRMZ3).

B. Sterilization of surface panicle:

Panicles were sterilized by dipping in chlorox 20% (Sodium hypochlorite 5.25% W/V) solution for 20 minutes and rinsed with sterile distilled water.

C. Dissection and culture:

The lemma of spikelets was cut as half to two thirds using sterilized scissors. The anthers from spikelets were picked using forceps and inoculated to the surface of Agar medium. 60 anthers were inoculated per/Petri dishe (60 x 15 mm), then seeling by parafilm and incubated under the dark at 26 ± 1°C.

When calli were formed and developed to 2 mm in diameter, calli were transferred to the differentiation medium.

D. Data collection:

1. The callus frequencies were calculated by using following equation.

$$= \frac{\text{No. of anthers forming callus x 100}}{\text{No. of inoculated anthers}}$$

2. The plant regeneration frequencies

$$= \frac{\text{No. of responsed callus for plant regeneration x 100}}{\text{No. of inoculated callus}}$$

3. No. of regenerant plants from each callus were recorded.

4. Plantlet height of regenerants after one month from sub culture was measured.

5. Callus diameter using graph paper was estimated.

E. Statistical Analysis:

Data on callus and regeneration frequencies were analyzed by Chi-square test to provoke the effect of different factors, genotypes and growth regulators, on response for callus induction and plant regeneration as well as for the independence of these factors on the response of anthers induction of callus and plant regeneration. Factorial analysis was applied in this study with two factors, genotypes and growth regulators, for studying their interaction (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Androgenesis of rice anthers developed into callus after 4-8 weeks showed different growth rate depending upon the genotypes and concentrations of hormones (Figure 1). After 4 weeks from emergence of callus, when the callus arrived into optimum stage for transferring to plant regeneration media, many calli were differentiated into plantlets. These plantlets exhibited differences according to the genotype and the medium (Figure 2).

Varietal response for callus induction under different concentrations of hormones were recorded (Table 2) and significant differences between varieties were shown for callus induction frequencies. Sakha 101 and Sakha 103 possessed higher frequencies than the other varieties, while moderate frequencies of callus induction were produced by Sakha 104 and Giza 177. In contrast, low frequency of callus appeared at Sakha 102 variety. These results are in agreement with many investigators (Aruna and Reddy, 1988; Ranjan *et al.*, 1988, and Yang *et al.*, 2001). Shahnewaz *et al.* 2004 studied the effect of genotype on induction of callus using six rice cultivars. Callus induction frequency varied from 1.42 – 8.06% depending on the cultivar. Highest callus induction was observed in BRRI Dhan- and lowest was observed in PIR.

With regard to the effect of hormone balances, all modified concentrations (CIMZ1, CIMZ2 and CIMZ3) gave callus induction higher than the control, (CIM0). Good concentrations of CIMZ2 and CIMZ3 gave more than twice the control for callus induction (Table 2). Therefore, CIMZ2 is considered as an improved hormone balance for increasing efficiency of anther culture

response of these genotypes under study. Moreover, highly significant differences of interaction between genotype x media composition (hormone concentrations) were recorded for callus frequences. Interested findings were recorded in interaction between Sakha 101 variety with CIMZ3, as well as Shaka 104 x CIMZ2 giving 27.5 and 25.0 callus frequencies, respectively.

These results are in the same trend were confirmed with many investigation on anther culture response in rice (Aruna and Reddy, 1988; Rout *et al.*, 1991; Panthee *et al.*, 1998; Yang *et al.*, 2001 and Islam *et al.*, 2004). Nguyen *et al.* (1993) studied the effect of interaction between genotype and culture medium on callus induction of rice anther culture using 12 genotypes and 3 different callus induction media. The genotype-media interaction was reported to be significant and callus induction ranged from 1.8% for cross SR 64 x SR 2588-1- 3.2 on G1 medium, to 28.0% for Nep Hoa Vang on L_8 medium. L_8 medium gave the highest callus induction (19.5%) followed by G_1 and Fj media.

Percentage of plant regeneration of different varieties under different regeneration media, PRMZ0, PRMZ1, PRMZ2 and PRMZ3, are presented in Table 3. Significant differences were shown between genotypes and growth regulator concentrations. Interaction between genotypes x hormone concentrations were highly significant for plant regeneration frequency (Table 3). High frequency of plant regeneration was recorder of Sakha 101 (61.95), followed by Sakha 104 (56.23). Sakha 102 gave lowest frequency from plant regeneration (33.33). Surprisingly Sakha 102 gave the lowest frequency over all concentrations, however, possessed higher frequency of plant regeneration (100%) under PRMZ2 medium. These results might throw the lights on the importance of interaction between this specific genotype and especial hormone blance (PRMZ2). In general, the present results recommended that PRMZ2 medium was an excellent medium for plant regeneration over all genotypes.

The above results are in agreement with these reported by many investigators, clearing the importance of genotypes and hormones balance for success of differentiation of anther culture derived callus (Rout *et al.*, 1991; Nguyen *et al.*, 1993 and Laxmi and Reddy, 1997).

Aruna and Reddy (1988) studied genotypic differences in plant regeneration from anthers of 11 indica rice varieties under 6 different media. Varietal response depended on the medium used. Regeneration of green plants occurred at a high frequency in Rasi (47.5%), PTB33 (35%) and TC (C) (10%). Of PTB33 regenerants, only two were haploid. In another study on the effect of plant growth regulators in callus induction and plant regeneration in rice anther culture, Islam *et al.* 2004 reported the regeneration of plants from callus on agarified MS medium supplemented with 0.5 mg/l alpha NAA. and 3 mg/l. Kinten was variable and ranged from 16.7% to 69.3%. The derived calli showed better performance for plant regeneration (69.3%) and among those plants, 56.14% were green.

Analysis of variance of some criteria belonging to anther culture, callus diameter, no. of plant regenerants and regenerant plant height are shown in Table 4.

Highly significant differences of genotype (A), media composition (B) and interaction between them (A x B) were detected for no. of regenerants per callus and regenerant plant height, while callus diameter possessed significant at interaction A x B only. These results explore that the efficiency of anther culture should include extensive studies on different genotypes and different hormone balances. Little effect of genotype and media composition on callus diameter was recorded, while interaction between them could be considered.

Sakha 101 and Sakha 104 possessed significant value of callus diameter under N_6 + 1mg/l NAA + 1mg/l 2ip medium (Table 5). Significant value of the latter medium for No. of regenerauts and plant height of regenerants. Therefore, PRMZ2 medium is considered as a good plant regeneration medium for these genotypes. Sakha 101 and Sakha 104 varieties possessed significant values under the latter medium for No. of regenerants and plant height of regenerants (Tables 6 and 7). It is obvious that the somaclonal variation in plant height of regenerants can play a role for determination of criteria performance and it requires more extensive study at the nearly future. Somaclonal variation was studied through several investigations. Sugimote *et al.* (1999) recorded the somaclonal variation in heading date and protein content in brown rice.

In general, the present study stated that (1) the interaction between genotype and media composition especially hormone concentrations could play an important role in anther culture response (2) therefore, the modern rice varieties might require more combinations of hormone balance for the discovery of their genetic importance, which can give high efficient anther culture responses and, subsequently, using anther culture for the genetic improvement of rice.

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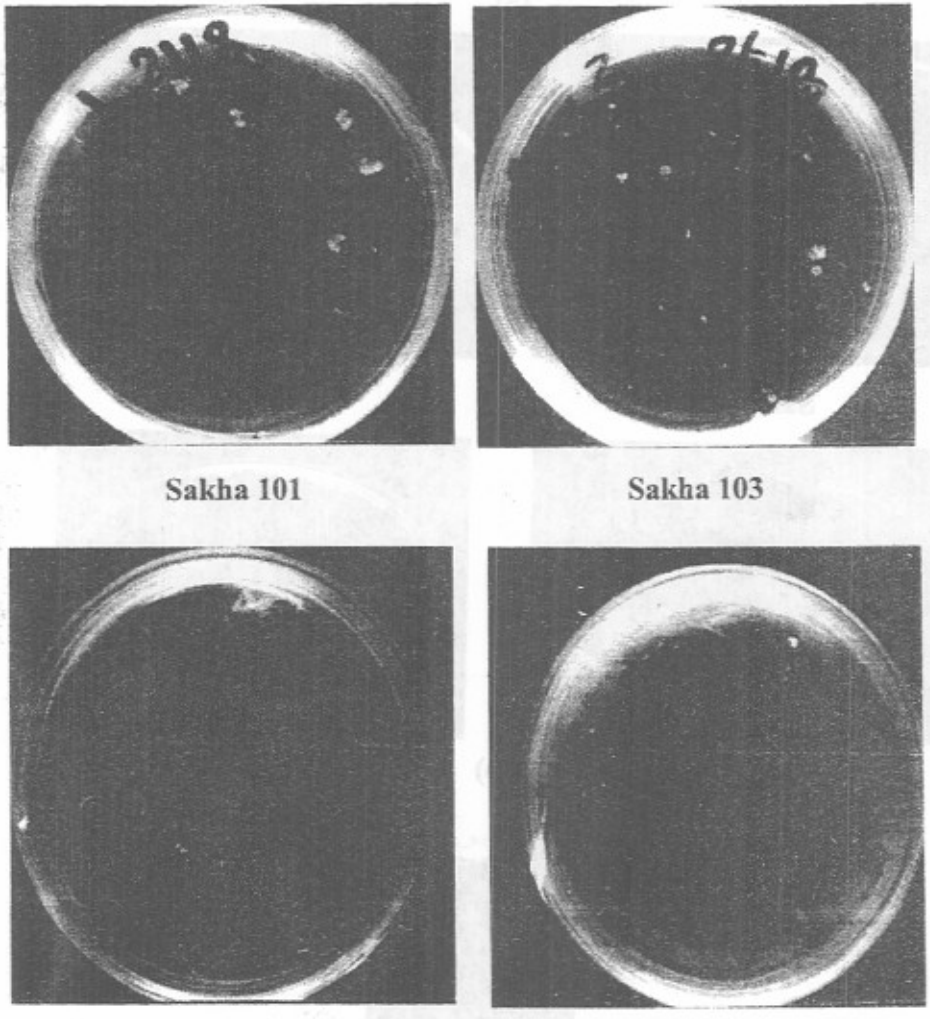

Fig. 1: Response of callus formation for studied varieties, after four weeks from planting

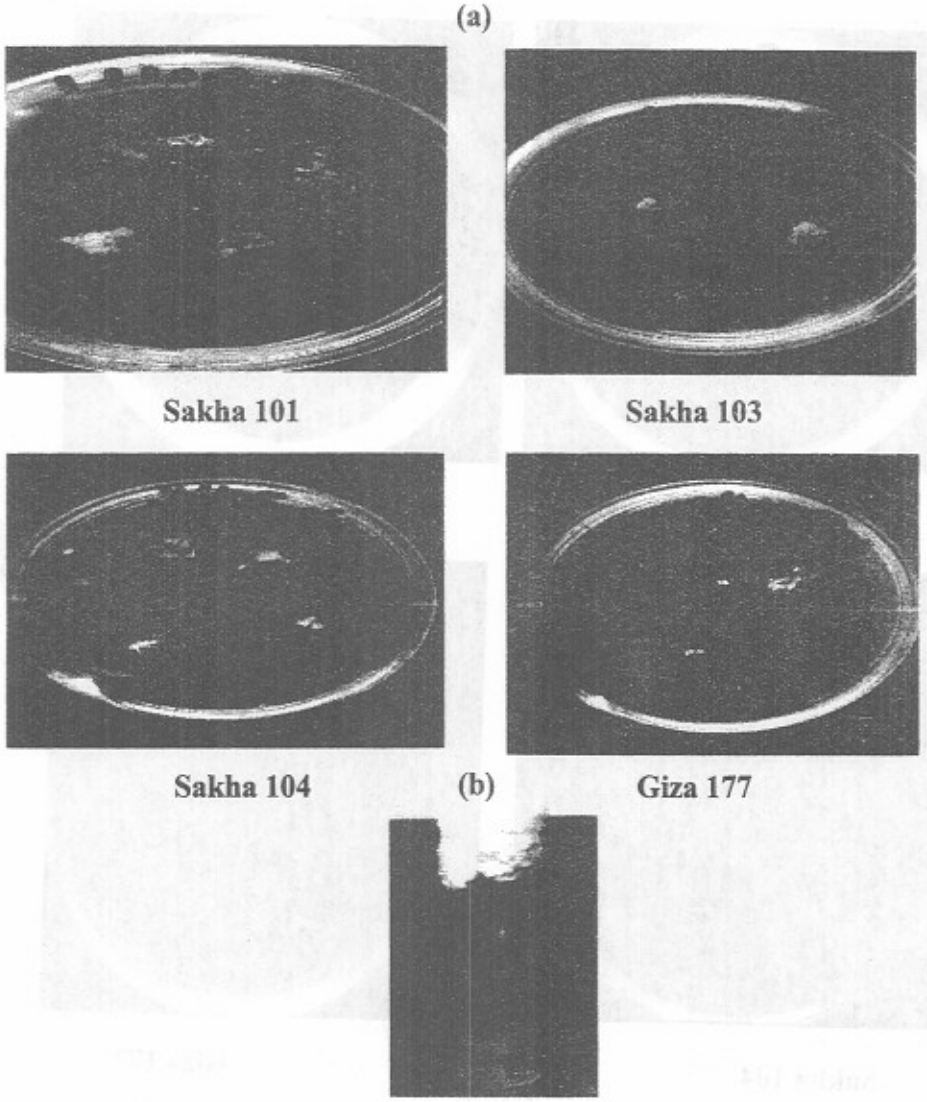

Fig. 2: (A) Response of plant regenerations for studied varieties, after four weeks from transferring of callus to plant regeneration medium and (B) plantlet from Sakha 104

Table 1: The origin and characterization of the studied varieties

No	Entry	pedigree	Origin	Type	Salinity tolerance
1	Giza 177 (P_1)	Giza 171/ Yamji No 1/Pi No. 4	Egypt	Japonica	Susceptible to salinity and drought
2	Sakha 101 (P_2)	Giza 176 / Milyang 79	Egypt	Japonica	Moderate
3	Sakha 102 (P_3)	GZ 4096-7-1/ Giza 177	Egypt	Japonica	Sensitive
4	Sakha 103 (P_4)	Giza 177/Suweon 349	Egypt	Japonica	Sensitive
5	Sakha 104 (P_5)	Gz 9640-8-1/ Gz4100-9-1	Egypt	Japonica	Salt tolerant and Moderate drought

Table 2: Chi-square test of callus induction frequency from anther culture of five rice genotypes on N_6 and B_5 media containing different concentrations of growth regulators

Media \ Genotypes	Sk 101	Sk 102	Sk 103	Sk 104	G 177	Mean
CIM0: N_6 + 2mg/L NAA + 0.5 mg/L kn.	10.00	0.00	10.00	2.00	1.70	4.74
CIMZ1: N_6 + 0.5 mg/L NAA + 1mg/L 2ip.	14.20	7.20	13.80	8.90	5.80	9.98
CIMZ2: N_6 + 1 mg/L NAA + 1mg/L 2ip.	16.70	10.60	20.00	25.00	8.30	16.12
CIMZ3: B_5 + 2.5 mg/L NAA + 0.5 mg/L kn + 2.5 mg/L 2,4 D.	27.50	0.00	25.00	13.30	21.30	17.42
Mean	17.10	4.45	17.20	13.05	9.28	

1. Genotypes
   $X^2c = 9.72$ $X^2t = 9.48$
2. Growth regulators
   $X^2c = 8.54$ $X^2t = 7.82$
3. Independence
   Genotypes x media
   $X^2c = 32.9$ $X^2t = 31.14$

Table 3: Chi-square test of plant regeneration frequency of five rice genotypes on MS medium containing different concentrations of growth regulators

Media \ Genotypes	Sk 101	Sk 102	Sk 103	Sk 104	G 177	Mean
PRM0: MS + 0.5mg/L NAA + 2 mg/L kn.	50.00	0.00	33.33	50.00	33.30	33.33
PRMZ1: MS + 0.5 mg/L NAA + 1mg/L kn + 1 mg/L 2ip.	62.50	33.33	68.70	41.60	66.66	54.56
PRMZ2: MS + 0.5 mg/L NAA + 0.5 mg/L kn + 0.5 mg/L 2ip.	66.66	100.00	43.70	66.60	50.00	65.38
PRMZ3: MS + 1 mg/L NAA + 4 mg/L kn.	68.70	0.00	58.30	66.60	33.30	45.38
Mean	61.95	33.33	51.00	56.23	45.08	

1. Genotypes
   $X^2c = 9.76$ $X^2t = 9.48$
2. Growth regulators
   $X^2c = 11.19$ $X^2t = 7.82$
3. Independence
   Genotypes x media
   $X^2c = 212.38$ $X^2t = 31.14$

Table 4 :Analysis of variance of callus diameter, no. of plant regenerants per callus and height of plant regenerants after one month from sub culture of four rice genotypes with three levels of growth regulators

S.O.V	d.f.	Callus diameter	No. of plant regenerants	Regenerant plant height
Rep.	2	0.06	15.26*	13.00
Treat.	11	1.03	269.64**	103.27**
Genotypes "A"	3	0.25	524.67**	202**
Media "B"	2	2.215	247**	93.25**
A x B	6	1.03*	149.67**	57.25**
Error	22	0.47	2.61	6.72

* = Significant at 5% level
** = Significant at 1% level.

Table 5: Means of callus diameter (mm) after one month from anther culture for four rice genotypes with three different concentrations of growth regulators

Media \ genotypes	Sk 101	Sk 103	Sk 104	G 177	Mean
CIM0 : N6 + 2 mg/L NAA + 0.5 mg/L kn.	1.50	2.33	1.50	2.00	1.83
CIMZ2: N6 + 1mg/L NAA + 1 mg/L 2ip.	2.66	1.33	2.83	2.50	2.33
CIMZ3: B5 + 2.5 mg/L NAA + 0.5 mg/L kn + 2.5 mg/L 2, 4D.	1.93	1.50	1.50	1.66	1.64
Mean	2.03	1.72	1.94	2.05	

$L.S.D_{0.05} = 0.99$
$L.S.D_{0.01} = 1.35$

Table 6: Means of No. of plant regenerants per callus of four rice genotypes with three different concentrations of growth regulators

Media \ genotypes	Sk 101	Sk 103	Sk 104	G 177	Mean
PRM0: MS + 0.5 mg/L NAA + 2 mg/L kn.	31.00	22.00	9.00	14.00	19.00
PRMZ2: MS + 0.5 mg/L NAA + 0.5 mg/L kn + 0.5 mg/L 2ip.	46.00	25.00	25.00	14.00	27.00
PRMZ3: MS + 1 mg/L NAA + 4 mg/L kn.	22.00	21.00	18.00	21.00	20.00
Mean	33.00	22.00	17.00	16.00	

$L.S.D_{0.05} = 2.73$
$L.S.D_{0.01} = 3.72$

Table 7: Means of plant height (cm) for regenerants after one month from subculture of four rice genotypes with three different concentrations of growth regulators

Media \ genotypes	Sk 101	Sk 103	Sk 104	G 177	Mean
PRM0: MS + 0.5 mg/L NAA + 2 mg/L kn.	30.00	33.00	15.00	25.00	25.75
PRMZ2: MS + 0.5 mg/L NAA + 0.5 mg/L kn + 0.5 mg/L 2ip.	35.00	25.00	21.00	26.00	26.75
PRMZ3: MS + 1 mg/L NAA + 4 mg/L kn.	25.00	22.00	21.00	18.00	21.50
Mean	30.00	26.50	19.00	23.00	

$L.S.D_{0.05} = 4.37$
$L.S.D_{0.01} = 5.95$

استجابة أصناف الأرز المصرية الحديثة لزراعة المتوك

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قسم الوراثة – كلية الزراعة – جامعة الزقازيق

تهدف هذه الدراسة إلى الكشف عن مدى استجابة أصناف الأرز المصرية الحديثة سخا ١٠١، سخا ١٠٢، سخا ١٠٣، سخا ١٠٤ و جيزة ١٧٧ لزراعة المتوك وكذلك تحديد أنسب توازن هرمونى لزيادة القدرة على إنتاج كالوس وإنتاج نباتات جديدة. وقد وجدت اختلافات كبيرة فى نسبة استحداث الكالوس بين البيئات المختلفة. تراوحت بين ٤,٧٤% إلى ١٧,٤%. ولقد أعطت بيئة CIMZ2 التى تشتمل على (N_6 + 1mg/l NAA + 1mg/l 2ip) وكذلك بيئة CIMZ3 التى تشتمل على (B_5 + 2.5mg/l NAA + 0.5 mg/l kn + 2.5 mg/l 2,4D) أعلى تكرار فى إنتاج الكالوس (١٦,١٢ – ١٧,٢% على التوالى). ولقد أعطى كلا الصنفين سخا ١٠١ و سخا ١٠٣ أعلى تكرارين بالنسبة للاستجابة لإنتاج كالوس عن الأصناف الأخرى فى مختلف التركيزات الهرمونية.

كما اختلفت القدرة على إنتاج نباتات جديدة من الكالوس على بيئة M.S اختلافاً كبيراً تراوح بين ٣٣,٣٣% إلى ٦٥,٣٨% حيث أعطت بيئة PRMZ2 التى تشتمل على(MS + 0.5 mg/l NAA + 0.5 mg/l kn + 0.5 mg/l 2ip) أعلى تكرار فى استحداث نباتات جديدة (٦٥,٣٨%).

ولقد أظهر الصنف سخا ١٠١ قدرة أكبر على استحداث نباتات جديدة عن باقى الأصناف بصرف النظر عن التركيزات الهرمونية. وعلى الرغم من أن الصنف سخا ١٠٢ أعطى أقل نسبة لاستحداث نباتات جديدة لمعظم التركيزات الهرمونية إلا أنه أعطى أعلى نسبة (١٠٠%) لإنتاج نباتات جديدة على بيئة PRMZ2 وهذا يبين أهمية التفاعل بين التركيب الوراثى والإتزان الهرمونى المناسب.

كما أمكن تعيين اختلافات عالية المعنوية بالنسبة للتراكيب الوراثية وتركيب البيئات والتفاعل بينهما فى كل من عدد النباتات الناتجة من كل كالوس وطول النباتات الجديدة، أما بالنسبة لحجم الكالوس فقد كان معنوياً بالنسبة لتفاعل التركيب الوراثى مع البيئة فقط. لذلك يلزم إجراء محاولات عديدة لتحديد أنسب المستويات الهرمونية لكل تركيب وراثى نظراً لأهمية التفاعل ما بين التركيب الوراثى والبيئة.