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GENETIC STUDIES ON RICE (*Oryza sativa* L.)

a- GENOTYPE AND HORMONAL EFFECTS ON CALLUS FORMATION AND REGENERATION

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ABSTRACT

Callus induction and green plant regeneration at high frequencies from Egyptian rice varieties Giza 172, 175, and 181 have been detected by coordinating the growth regulators in the induction medium. Two cytokinins 6- Benzyle amino purine (6- BAP) and kinetin at three concentrations of each (1, 2, and 3 mg/L were used in combinations with four auxins 2,4-dichlorophenoxy acetic acid (2,4-D), 1-H Indole-3- acetic acid (IAA), 1-Naphthalene acetic acid (NAA), and Indole butyric acid (IBA) at two concentrations of each (0.1 and 0.5 mg/L).

Giza 172 scored the highest frequency of callus induction in the presence of 6-BAP (2 mg/L) combined with 2,4-D (0.5 mg/L) while, 2 mg/L kinetin and 0.4 mg/L NAA gave rise to higher number of shoots / callus. Meanwhile Giza 175 responded better for callogenesis on medium contained 6-BAP (1 mg/L) and 2,4-D (0.1 mg/L) and showed good response for shooting in the presence of kinetin only (1.0 mg/L).

On the other hand, Giza 181 showed higher presence for callus induction on the combination of 1 mg/L 6-BAP with 0.5 mg/L 2,4-D while, shoots regenerated / callus were better in the presence of 4 mg/L. Kinetin with 0.1 mg/L NAA.

All the three tested varieties showed positive response for callogenesis in the presence of 2,4-D combined with 6-BA and the morphoyenesis response was in the presence of kinetin with NAA.

It appears that, in spite of substantial genotypic variability exists in cultivated rice, they have the potential for tissue culturing. Therefore, selection for desirable rice traits in vitro would be facilitated.

INTRODUCTION

There are several reports on callus initiation and plant regeneration in rice. (Abe and Sasahara, 1982, and Heyser *et al.*, 1983).

Some of other studies on rice tissue cultures have been conducted concerning the effect of exogenous hormones. (Henke *et al.*, 1978, and Cornejo-Martin *et al.*, 1979). Inspite of different explants that contain immature meristematic cells develop callus that is competent to express totipotency, especially in monocots, the difficulties encountered in obtaining the desired response from an explant are numerous. (Bhaskaran and Smith 1990).

Systematic searches for appropriate genotype-culture environment combinations is necessary toward the practical application of *in vitro* genetic manipulation in rice breeding especially those adapted to Egypt. Therefore, we conducted this research to investigate the response of some Egyption rice genotypes to *in vitro* growth conditions and detect the best hormonal concenterations to initiate, maintain and regenerate plants from callus tissues.

MATERIALS AND METHODS

Three Egyptian varietis of rice namely Giza 172, Giza 175, and Giza 181 were used in this study. Mature seeds were selected and dehusked. Seeds were sterilized in 70% ethanol for 30s and in 30% clorax for 30 min, followed by three rinses with sterile distilled water. The sterilized seeds were placed on the surface of an agar meduim cantaining salts as in Murashige and Skoog's meduim (1962). Seeds

were incubated at 28°C in dark for 24 h till germination. Germinated seeds were transferred to plates containing N6 (Chu *et al.*, 1975) meduim for callus induction. Formed calli were isolated from scutellum of mesocotyle from the original seeds. The capacity for callus formation were evaluated by weighing four calli in each variety.

Calli for plant regeneration were maintained by continuously selecting and transferring regenerable calli during maintenance of the culture on N6 medium. Callus induction media were supplemented with either kinetin or 6-Benzyle amino purine (6. BAP) at three different concenterations 1,2, and 3 mg/L. in combination with four auxins, 2,4 - dichlorophenoxy acetic acid (2,4-D), 1- Naphthalen acetic acid (NAA), IH-Indol-3- acetic acid (IAA), and Indole Buteric acid (IBA) at two concenterations 0.1 and 0.5 mg/L. Cultures were maintained on the growth media supplemeuted with yeast extract 500 mg/L, casein hydrolysate 500 mg/L, glutamine 100 mg/L and glycine 50 mg/L. for 9 weeks until we transferred them to shoot initiation media.

Culture conditions :

i. Callus induction

Callus cultures incupated at 28°C and photoperiod 6 hrs. for 9 weeks.

ii. Shoot induction

Shoot induction was done at 30°C and photoperiod 16 hrs. for 12 weeks.

iii. Root induction

Roots formed after 3 weeks at 30°C and photoperiod 8 hrs.

Shoot initiation media supplemented with kinetin (1,2 and 4 mg/L) and NAA (0.1, 0.4 and 1.0 mg/L.) and casein hydrolysate at 200 mg/L and glutamine at 50 mg/L. Regenerated plantlets were transplanted into small pots containing peatmoss. Pots were covered with plastic bags for 10 days to maintain high humidity and then gradually opened. The experiment was arranged in factorial design and data were analyzed statistically according to Snedecor and Cochran (1979).

RESULTS AND DISCUSSION

Results revealed that Egyptian rice genotypes can be manipulated *in vitro* for callus induction and subsequent green plant regeneration by testing their responses to various growth regulator combinations. It appeared that rice variety Giza 181 has the tendency for multiple shooting from clusters on the calluses. comparing to Giza 175 and 172.

This cultivar showed greater morphogenetic capacity both in respect of somatic-embryogenesis and shoot formation. The highest percentage of callus production effeciency (70 and 80%) was detected for Giza 172 and Giza 181 respectively in media containing 2.4-D at 0.5 mg/L and 6-BAP, (Table 1). Meanwhile Giza 175 showed 75% Callus production effeciency at 0.1 mg /L 2,4 -D and 1 mg/L 6-BAP. Despite the fact that all the tested three varieties were able to form calli in the presence of NAA at either 0.1 or 0.5 mg/L in combination with 6-BAP at various concentcation, the effeciency is very low

comparing to 2,4 -D presence in the growth media. This finding is contractions with what Rout and Sarma (1991) reported in callus induction in rice anther culture. Table (1) also showed the genotypic differences between the three tested varieties. In general Giza 181 showed better effeciencty for callus induction than Giza 172 and Giza 175. In fact, this genetic variation was detected earlier in rice (Abe and Futsuhara 1986).

It should be mentioned that the those tested rice varieties did not respond or produce calli at all on media containing combinations of IAA with 6-BAP and IBA with 6BA at various cencentoations (data not shown). All calli that produced in the presence of 2,4-D failed to regenerate. This finding supported what Rout and Sarma (1991) have been concluded that auxins in the induction media had considerable influence on the regeneration capacity of the interspeific hybrid calli in rice.

Once more, genotypic variable response toward callus production is pronounced (Table 2). In a trail to detect the best growth regulator combinations for Egyptian rice varieties, Giza 175 showed the most positive response as it scored 50% callus induction effeciency in the presence of 2,4 - D at 0.1 mg/L combined with kinetin at 2 mg/L, whereas Giza 181 showed the lowest response (15%) at the same hormonal combination. On the other hand, Giza 172 scored the highest callus production effeciency (40%) on N6 media centaining combination of NAA and kinetin at 0.1 mg /L and 2 mg/L respectively. Both Giza 175 and Giza 181 scored lower effeciency at the same hormonal combinations, but similar (25%).

Table (1) : Genotypic response (as % effeciency of rice callus induction production) to 6 BAP and various auxins at different concentration as percentages.

Auxins mg/L		6 BAP mg / L.								
		Giza 172			Giza 175			Giza 181		
		1	2	3	1	2	3	1	2	3
2,4-D mg/L										
	0.1	50	20	15	75	25	25	55	15	15
	0.5	40	70	35	35	40	20	80	45	40
NAA										
	0.1	35	40	15	30	45	20	35	20	20
	0.5	30	35	20	30	35	15	25	25	20

Table (2) : Effeciency of rice callus production on N6 media containing kinitine and various auxins (2, 4-D, IAA, and NAA), at different concentrations as percentages.

Auxins mg/L.		Kinetin mg/L.								
		Giza 172			Giza 175			Giza 181		
		1	2	3	1	2	3	1	2	3
2,4-D	mg/L									
	0.1	0	40	20	-	50	45	-	15	30
	0.5	15	25	15	20	15	10	30	20	15
IAA	0.1	10	15	20	15	20	5	10	5	15
	0.5	0	0	0	0	0	0	0	0	0
NAA	0.1	30	40	20	35	25	20	20	25	30
	0.5	10	15	5	5	10	5	5	20	15

The three tested varieties were not able to form calluses in the presence of IBA at all and this depressive affect was noticed also for IAA, inspite of some successful calli (were not able to continue). (data not Shown). This finding supported by what Ozawa and Komamine (1989) reported that hormonal concentrations had notable effects on differentiation. They found that rice callus can grow but did not differentiate in media supplemented with auxins and cytokinins at high concenterations Fig. (1) represent steps of callus formation maintenance of the three tested rice varieties.

In fact, contrasting data on the need for added cytokinins for the regeneratien production could be due to different levels of endogenous cytokenins depending on different plant genotype.

Table (3) showed the analysis of variance for used rice genotypes and growth regulators and their effects on the frequency of shoot induction. There were highly significant differences in this aspect among the used Egyptian varieties (Fig. 2). In fact interaction of genotype and treatment were highly significant and centributed most variation obtained in the analysis of variance, demonstrating that genotypes perfrom differently. This finding supports previous reports in rice anther culture by Quimio and Zapata (1990) and Rout and Sarma (1991), and in callus fromation and plant regeneration in rice tissue culture (Abe and Futsuhara 1986).

Rice variety Giza 175 appeared to be the most responsive variety toward rooting regardless of the growth regulator constituents, though there is some variation in number depending on the hormonal cencentration (Table 4 and Fig. 3).

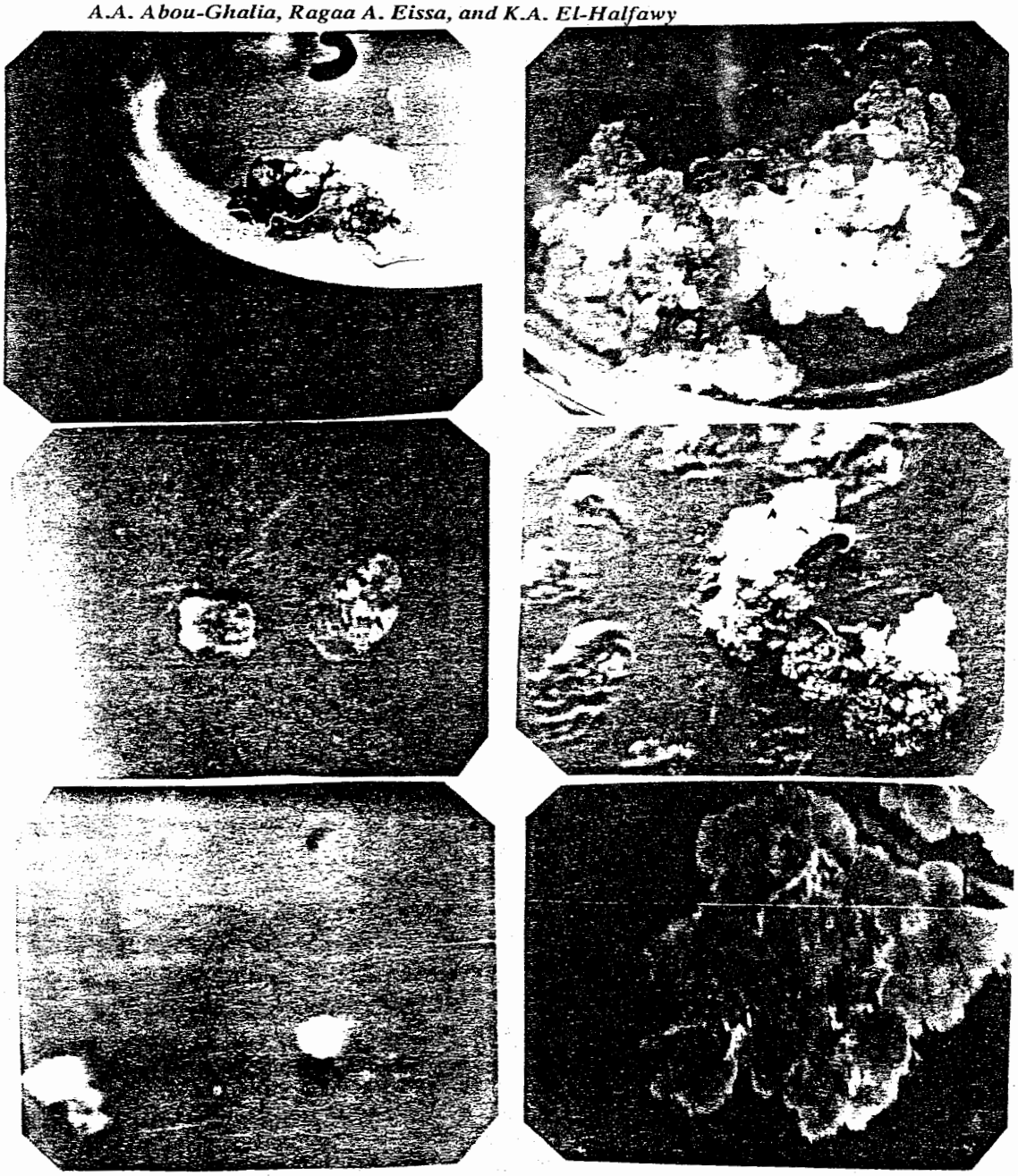


Fig. (1) : Comparison between the three tested rice Egyptian varieties, Giza 172 (a), Giza 175 (b) and Giza 181 for cullus formation and maintenance on N6 nedia supplemented with 6-BA and 2.4-D at various concentrations. The 2 ages of cullus growth over 3 wks period.

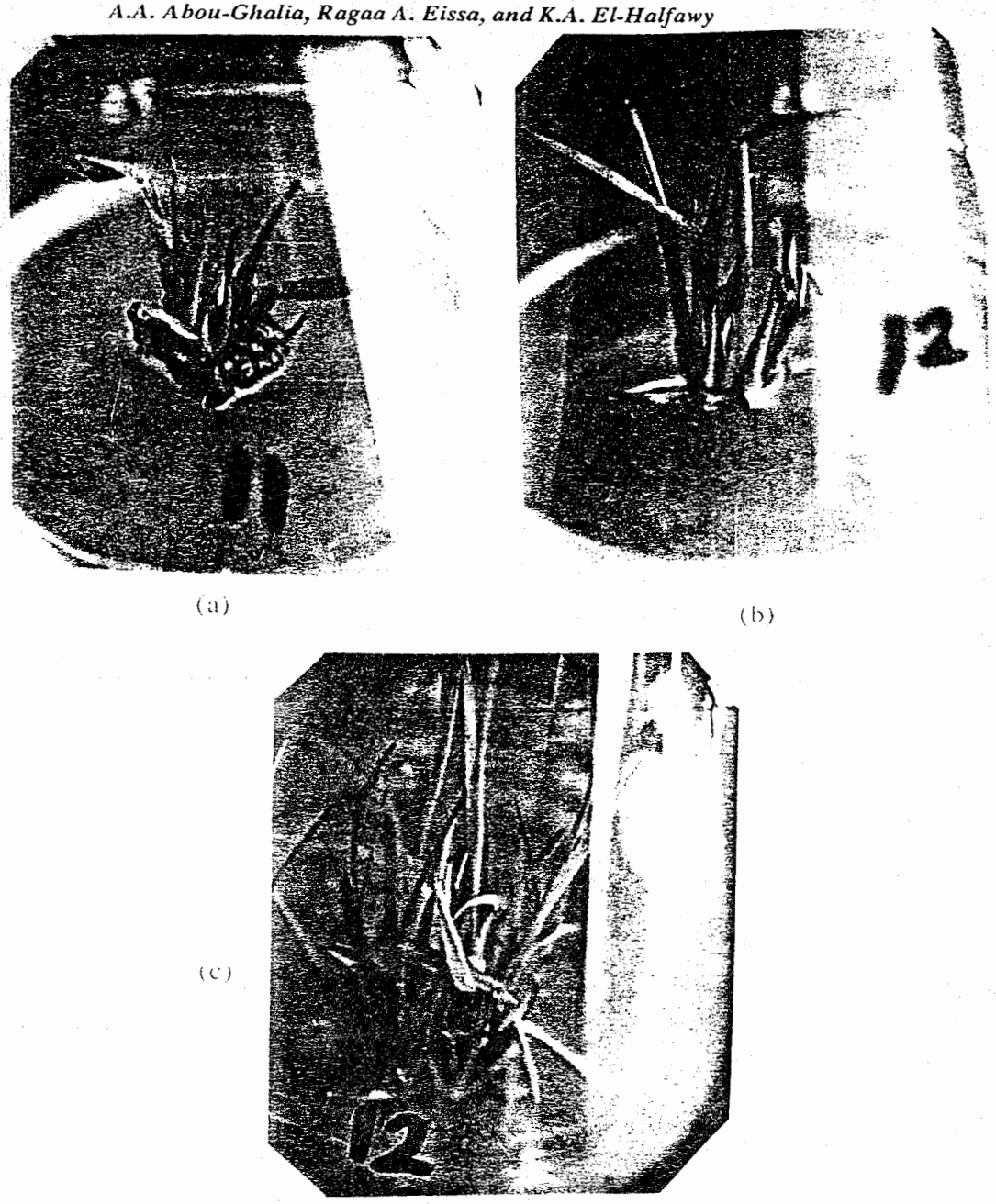
Table (3) : Analysis of varaince for the effects of genotypes and growth regulator combinations on the frequency of shoot induction.

Source of variation	DF	SS	MSS	F
Replication	2	1.05	0.525	1.02
Variety	2	204.39	102.295	198.4**
Repvar.	4	2.06	0.515	800.7**
Treatment	11	6077.36	552.500	908.3**
Var X Treat.	22	13786.50	626.700	
Error	66	55.54	0.690	

** Highely significant at 1 % level.
L.S.D.

Table (4) : Mean numbers of roots developed on rice varieties, Giza 172, 175, and 181 after 10 days of callus transfer on rooting media supplemented with NAA, IAA and IBA at indicated concentrations in combination with 2 mg / L Kinitinee.

	Homone	conce.	Varieties		
			Giza 172	Giza 175	Giza 181
a	NAA	1mg/L.	2.30	4.00	1.67
b		2	4.00	5.00	1.30
c		3	0.30	2.30	2.30
d	IAA	1mg/L	1.670	3.67	1.67
e		2	1.34	1.30	2.67
f		3	1.30	1.67	1.30
G	IBA	1mg/L	2.30	3.30	1.00
h		2	4.30	4.30	1.67
i		3	2.00	2.00	1.30



(a) (b)

(c)

Fig. (2) : Plantlets regeneration from cullus of rice varieties, Giza 172 (a), Giza 175 (b) and Giza 181 (c) on N6 medium suplemented with kinctin and NAA at various concentrations (at 10 weeks of age).

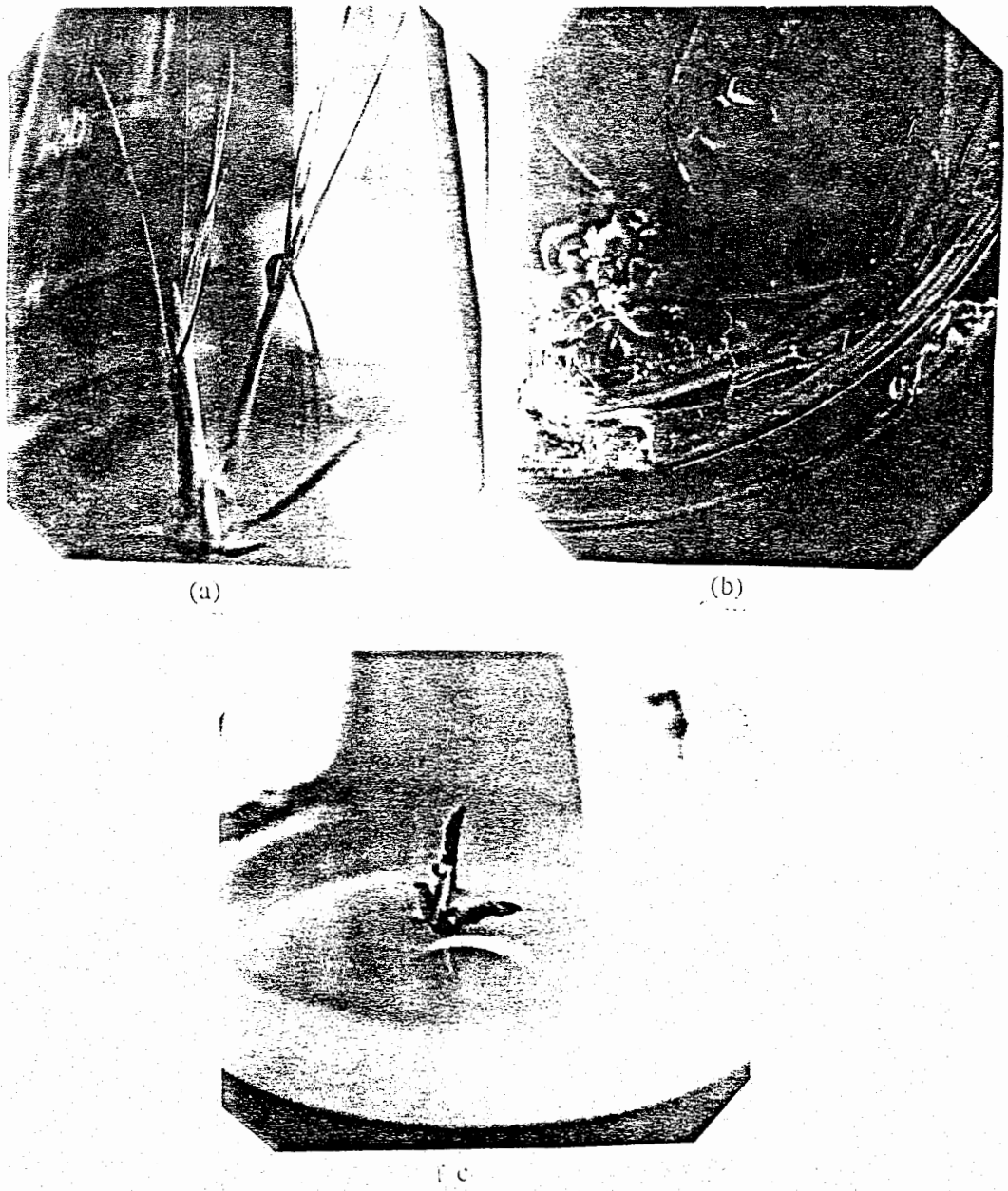


Fig. (3) : Rice varieties, Giza 172 (a), Giza 175 (b) and Giza 181 (c) on N6 medium at three weeds of age.

On the contrary was the variety Giza 181, differing from both varieties. Giza 172 and Giza 175 showed the highest number of roots induced upon the presence of NAA, IAA or IBA at 2 mg/L Combined with kinetin at 2 mg/L. There is no doubt that rice genotype exerts a major infleuence on the *in vitro* performance. These conclusion have been stated by Abe and Futsuhara (1984 and 1986). Fig. (1, 2 and 3) represent steps of callus maintenance and plant regeneration of Egyptian rice varieties.

These figures clearly illusterate the capability of adapted Egyptian rice varieties for growth and reproducability from tissues in culture.

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الملخص العربى

دراسات وراثيه على الأرز
أ – تأثير التركيب الوراثى والهرمونات على تكوين مزرعه الانسجه واستعاده تكوين النبات الكامل منها.

عاطف أبو غاليه ، رجاء عيسى ، خليل الحلفاوى
قسم الوراثه كليه الزراعة ومركز زراعة الأنسجه والهندسة الوراثية جامعه المنوفيه.

استخدم فى هذا البحث ثلاثه أصناف أرز منزرعه فى جمهوريه مصر العربيه وهى جيزه ١٧٢، جيزه ١٧٥، جيزه ١٨١ لدراسة قدرتها على النمو فى مزارع الانسجه وكذلك تحديد أفضل نوعيه وتركيز منظمات النمو اللازمه لنموها النمو الأمثل.

استخدم نوعين من منظمات النمو (السيتوكينين) وهما ٦ بنزيل امينوبيورين والكينتين بثلاثه تركيزات مختلفه لكل منهما وهى ١ . ٢ . ٣ ملجم/لتر.

استخدم ايضا أربعة منظمات نمو (الاكسين) وهى ٢-٤ داى كلوروفينوكسى استيك أسيد، نفتالين أسيتيك اسيد ، اندول اسيتك اسيد وكذلك اندول بيوتريك اسيد لكل منهم ١ . ٠ ، ٠ . ٥ ملجم/لتر.

يتضح من النتائج ان صنف جيزه ١٨١ أعطى أعلى معدل لتكوين الكالوس فى وجود ٦ بنزيل امينوبيورين بتركيز ١ملجم/لتر مع داى كلوروفينوكسى استيك أسيد، بتركيز ٥ر٠ ملجم/لتر وايضا اعطى أعلى معدل فى تكوين المجموع الخضرى وكذلك اعاده تكوين نباتات من مزارع الانسجه.

بينما استجاب جيزه ١٧٢ أفضل فى بيئه تحتوى على ٢ ملجم/لتر ٦ بنزيل امينوبيورين مع داى كلوروفينوكسى استيك أسيد، بتركيز ١ر. ملجم/لتر. بينما جيزه ١٨١ سجل اعلى معدل فى اعاده تكوين النباتات فى وجود كينتين بتركيز ٤ ملجم/لتر و ٥ر٠ ملجم/لتر من اسيتيك اسيد .

وبالرغم من الاختلاف الوراثى الواضح في استجابه الاصناف المدروسه لتركيزات مختلفه من منظمات النمو الا أنها اثبتت قدره الاصناف المنزرعه علي النمو فى بيئه مزارع الانسجه بكفاءه مما يبشر بفرصه سانحه لدراستها وراثيا بإسهاب.