

BIOTECHNOLOGY AND RICE IMPROVEMENT IN EGYPT: APPLICATIONS, CONSTRAINTS AND PROSPECTS

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Abstract

Sustainable agriculture is the main challenge of the future. Food supplies will have to be more than double by 2025 to ensure sufficient quantity and quality, to meet the increase in population rate and demand.

Maximization of yield per unit area is the main target of rice breeders in Egypt. Fortunately, the breeders have developed the highest grain yield rice varieties but in order to face high population rate, the three main strategies are needed i.e. improvement of saline soil productivity, utilization of hybrid rice technology, full utilization of all technologies in rice production including modern biotechnology will play a decisive role in increasing yield to maintain sustainable locally self-sufficient in food for coming generations.

Rice biotechnology is becoming increasingly important as a tool for developing varieties with multiple resistance to pests and diseases, those which can use soil nutrients more efficiently, tolerate adverse soil stresses and are tailored for multiple cropping and high yield production per day.

Many areas of biotechnology proved their potentiality in rice breeding like tissue culture, haploid culture, embryo culture and rescue, genomic analysis, transformation through biolistic and *Agrobacterium* mediated transformation, gene tagging, marker assisted selection or molecular breeding and gene pyramiding, structural and functional genomics.

In Egypt, tissue culture especially anther culture technology gave a good promise for developing high grain yield lines; resistant to both blast and stem borer, those lines are : SK 101/IR 65829 (10.40 t/ha) and SK 101/Akiyutaka (9.75 t/ha).

Moreover, salt-tolerant double haploid lines were developed i.e, SK 102 / Hexi 30 // SK 101, SK 101/GZ 5844 // IR 65600-96, those lines can tolerate up to 10 ds/m.

Low amylose content and high tolerance to salinity were combined together in the background of the line AC 1225 (GZ 1368-5-4/Milyang 49). Moreover, this line had an allelopathic activity against *Cyperus difformis* and *Echinochloa crus-galli*. Some of developed AC lines were highly resistant to stem borer. On the other hand, a total of 271 PGMS lines were selected through AC technique to be utilized in hybrid rice production.

Regarding molecular marker techniques and their applicability in rice breeding. Protein and isozyme markers were practiced for discriminating among cultivars mainly for salinity and drought.

More work will be designed to widen the utilization of different molecular marker techniques in Egyptian rice breeding program.

INTRODUCTION

Rice is an important cereal and a main source of calories for more than one-third of the world population. Rice production was increased to more than 600 million tons after 2000. The increment in production has mainly been achieved through the application of conventional plant breeding methods coupled with new technologies. To meet the growing needs of the world population, which is likely to be 8.3 billion in 2025, 40% more rice is required. Mean challenges need to be faced like several biotic and abiotic stresses which severely reduce rice production (Persley and Lantin, 1999).

Biotechnology has become a major tool in solving an increasing number of fundamental and applied problems that concern plants and animals covering areas of cellular biology, biochemistry and physiology. Rice biotechnology is becoming increasingly important as a tool for developing varieties with multiple resistance to pests and diseases, those which can use soil nutrients more efficiently, tolerate adverse soil stresses and are tailored for multiple cropping and high yield per day; and high nutritional value. Many areas in biotechnology could be useful; tissue culture for clonal propagation and germplasm conservation, haploid culture technologies, somaclonal variation and cell culture screening, embryo culture and rescue (wide hybridization) , gene transfer and regulation and control of gene expression through recombinant DNA technique.

1- Tissue culture and rice improvement :

1-1: Anther culture :

This technique has been applied since 1968 in Japan by Nijzeki and Oono. Since then, this technique has been widely used in China, South Korea, Colombia, CIAT, IRRI, Hungary and Egypt (Heszky and Simon- Kiss, 1992, Draz *et al.*, 1992, 1994 and 1998). This technique has attracted wide attention because of the possibility for obtaining haploid cells, tissues, and complete organisms with only a single dose of genetic information. Mutants could be more readily discovered because only one set of chromosomes and only one copy of each gene are present in the haploids (Raina, 1989).

To fulfill the main breeding program objectives in Egypt i.e. high yielding ability, earliness (125 days), pest resistance, superior grain quality, and tolerance to soil stress conditions, rice breeding programs in Egypt have started practicing anther culture technique since 1992 to accelerate the breeding cycle by at least 3-5 years from the life cycle of the plant for the best selected crosses.

1.2- Main accomplishments by using anther culture technique :

1.2.1- Combining low amylose content and salt tolerance:

Anther culture has been used to transfer and combine tolerance to salinity with low amylose content by crossing the high amylose content and salt tolerant line GZ 1368-S-5-4 with Milyang 49 as low amylose content. Some lines were produced, tested and used as donors since 1995 (Table 1). The produced lines have low amylose content (18%), tolerant to salinity (4000 ppm), early and high in grain yield. Some of them were pushed to yield tests during 1993 and 1994 and the others were used as donors in the crossing program.

1.2.2- Anther culture lines possess allelopathic effects for controlling weeds:

Allelopathy means the production of allelochemicals, mostly secondary metabolites by rice plants that can affect the growth of weeds around the rice plant (Dilday *et al.*, 1994). These chemicals escape to the environment and influence the growth and development of another plant (Rizvi and Rizvi, 1992). Some weeds can reduce rice yields by 30% when competing with rice in a water seeded culture especially barnyardgrass (*Echinochloa crus-galli*). Therefore, new area was discovered in rice improvement through identifying the sources and donors for this character in Egypt. Screening method was developed, through weed infestation, radial area (cm) was measured, the percentage of weed control within the affected area based on the number of weed plant in the check plot around a control plant that had no apparent allelopathic activity to the weed.

Allelopathy results when living organisms produce bioactive molecules, which may in turn be modified, and these compounds enter the environment and produce direct or indirect effects on the growth and development of individuals of the same or other species.

Chemicals as secondary metabolites can be produced by rice against weeds at the seedling stage. The rice plant can kill rice weeds around the root zones by producing such chemicals. More than twenty compounds are the main chemicals affecting the weeds.

Different anther culture lines were developed and screened for the presence of this trait. From Tables (1 & 2) AC 1225 line obtained from the cross GZ 1368-5-4/Milyang 49 had allelopathic activity against barnyardgrass and *Cyperus difformis* with a percentage of 50% control of the weed species around rice roots (Tables 8 & 9). Moreover, the line AC 2541 had a 82.5 % control of *E-crus-galli* obtained from SK 102/Hexi 30//SK 101.

Table 1. Allelopathic activity of different rice varieties/lines around barnyardgrass.

Varieties / Lines	Origin	Control % (<i>E.crus-galli</i>)
AC 2541	Egypt	82.5
AC 1225	Egypt	50
E. Yasmine	Egypt	90
GZ 5310-20-2-2	Egypt	50
IET 1444	India	85
LD 183-3	Sri-Lanka	85
RP 2271-433	India	90
TKY 1014	Korea	60

Table 2. Allelopathic activity of different rice varieties/lines around *Cyperus difformis*

Varieties / Lines	Origin	Control %
Kim Rad F 87	Japan	90
Dular	Indica	60
Egyptian Yasmine	Egypt	90
AC 1225	Egypt	50

1.2.3. Increase the level of resistance to rice blast :

Utilization of tissue culture for pathology – oriented studies are many and various-lines developed through anther culture are immediately homozygous, resistance characteristics are fixed and no further segregation will occur. This is useful in transferring disease resistance to rice cultivars (Rush and Shao, 1981).

By using anther culture technique in Egypt, many lines resistant to rice blast caused by the fungal pathogen (*Pyricularia grisea* Sacc.) were developed. Blast caused severe losses (> 50%) during 1984. Since that time, efforts have been increased to control this disease by developing the resistance in the genotypes (Sehly *et al.*, 1998).

Rapid production is urgently needed to develop genotype- pathogen resistance through anther culture.

Successful achievements were obtained by producing dihaploid lines resistant to blast during vegetative (Leaf blast) and reproductive (neck blast) phases.

As shown from Table (3), a total of 375 lines were produced from different phenotypic acceptability from the breeding point of view. These lines were screened during seedling stage in blast nursery test and subjected to mixture of races presented in the 6 Governorates which grow rice in the northern part of the Delta.

Out of the 38 selected lines, about 25 lines were shown to be resistant under blast nursery test at the seedling stage. On the other hand, a greenhouse test was performed for specific virulent races of the fungus. About 18 lines proved to be resistant.

Table 3. Screened against blast disease

Crosses	Score of parents	No. of lines		No. of selected lines at each test					
		Total	Selected	Blast nursery			Greenhouse		
				R	M	S	R	M	S
Giza 177 x Zhong Hoa 3	R x R	84	18	7	2	9	4	7	7
Sakha 101 x BL 1	S x R	260	17	15	0	2	14	0	3
Giza 177 x Pi No. 4	R x R	10	2	2	0	0	0	2	-
GZ 4196-36/Zhong Hoa-3	R x R	21	1	1	0	0	0	1	0
Total		375	38	25	2	11	18	10	10

R= Resistant

M= moderately resistant

s= Susceptible

Another evaluation test was performed to intensify the tests to make sure of the resistance during both vegetative and reproductive phases (Table 4). A total of 27 lines were resistant during the vegetative stage. Moreover, only 19 lines proved to be resistant at the panicle stage (Table 4).

From the aforementioned results, it could be concluded that tissue culture for different F₁ crosses has been used as a quick tool for developing resistant lines against blast. These genotypes could be used either directly or could be used as sources and/or donors for resistance in the crossing program.

Table 4. Reaction of lines derived by anther culture from each cross for leaf and panicle infection under field conditions

Cross	Leaf infection			Panicle			Total
	R	M	S/SH	R	M	S	
Giza 177 x Zhong Hoa 3	11	6	1	8	-	10	18
Sakha 101 x BL 1	16	1	-	11	-	6	17
Giza 177 x Pi No. 4	-	2	-	-	-	2	2
GZ 4196-36/Zhong Hoa-3	-	-	1	-	-	1	1
Total	27	9	2	19	-	29	38

R = resistant, M = moderately resistant, S/HS = susceptible and high susceptible

1.2.4 – Anther culture lines resistant to stem borer :

Stem borers have become a major pest potentially limiting the production of rice. Tan *et al.* (1983) indicated that the density of adult *Chilo agamemnon* could affect the yield of paddy rice and has been found as destructive to rice fields especially indica types.

Anther culture laboratory at the Rice Research and Training Center has developed several lines with different useful traits .

Table (5) shows low values of white head percentages for anther culture lines which ranged from 2.10 to 3.89%.

Anther culture derived lines having nearly 2% of WH could be considered promising as sources of resistance to the borer. So, AC 1204, 1298, 1300, 1324, 1334 and 1338 could be utilized in such concern.

Table 5. Evaluation of some anther culture lines for rice stem borers *Chilo agamemnon* Bles. (1998)

No.	AC. No.	Parentage	White head (%)
1	-	Sakha 101	2.02
2	-	Sakha 102	4.33
3	1058	GZ 4120-205/K3	3.03
4	1126	Giza 176/IR 65598-112-2	3.89
5	1204	GZ 5470-14-1/IR 61633-B-2	2.00
6	1298	Sakha 102/TCCP 266-49-B	2.18
7	1300	Reiho/IR 65600-27-2-2	2.25
8	1324	GZ 4884-17-1/GZ 4596-3-3-2	2.15
9	1334	GZ 4884-17-1/GZ 4596-3-3-2	2.22
10	1338	GZ 4884-17-1/GZ 4596-3-3-2	2.10

1.2.5- Development of TGMS and PGMS through anther culture :

A total of 92 PGMS lines were developed by using anther culture technique. A total of 21 anther culture derived lines were developed from a cross between Sakha 101 and a Chinese variety were found to be uniform and completely sterile and are being studied for their fertility transformation. The best selected ones will be multiplied and utilized for test crossing next year.

1.2.6. Development of high salt-tolerant lines:

Four lines performed the best under saline soil conditions during 2003 season. Two of them surpassed the salinity tolerant variety Giza 178. The two lines AC 2624 and 2627 gave 6 and 6.1 t/ha, respectively (Table 6). Salt tolerance can be reached through haploid breeding.

Table 6. Performance of AC lines under saline conditions (2003)

No.	AC No.	Designation	Yield t/ha	Grain shape	Bl	SB
1	CK	Giza 178	4.3	Sh	R	R
2	2535	SK 101/IR 65829-2B-2R	4.5	Sh	R	R
3	2606	SK 101/ GZ 5890-26//SK 101	4.3	Sh	R	R
4	2624	GZ 5310-20/Hexi 24//GZ 5721-19	6	Sh	R	R
5	2627	GZ 5310-20/ Hexi 24// GZ 5721-19	6.1	Sh	R	R

ECe = 8

1.2.7. High grain yield developed lines :

Many lines have a high grain yield and good performance as well as resistant to blast and stem borer. The yield was higher than the check variety and

reached a maximum of 10.6 and 10.2 t/ha for AC 2602 and 2536 lines, respectively (Table 7).

Table 7. Performance of some AC lines during (2003 season)

No.	AC No.	Designation	Yield t/ha	Grain shape	BI	SB
1	CK	Giza 178	8.0	Sh	R (2)	R
2	2535	SK 101/IR 65829-2B-2R-4-P	9.9	Sh	R (2)	R
3	2536	SK 101/ IR 65829-2B-2r-4-P	10.2	Sh	R (2)	R
4	2546	Daey 2 Byeo / GZ 5385-29-3	9.9	Sh	R (4)	R
5	2577	SK 101/Akiyutaka//GZ 5721-19	9.6	Sh	R (2)	R
6	2602	SK 101/GZ 5990-26//SK101	10.6	Sh	R (2)	R
7	2627	GZ 5310-20/Hexi 24 //GZ 5721-19	9.5	Sh	R (2)	R
8	2639	GZ 5385-1/Gaori// GZ 5385-1	9.8	Sh	R (4)	R

2- Protoplast culture and fusion :

Attention for plant regeneration from protoplast of indica and japonica rice has been paid by many scientists. Yamada *et al.* (1985) were the first to regenerate plants from rice protoplasts. Since that time, many laboratories have regenerated plants of several japonica and indica cultivars. Yang *et al.* (1989) produced hybrid plants through the donor-recipient protoplast fusion method by electrofusing the gamma irradiated protoplast of A-58 CMS and the iodoacetamine-treated protoplasts of fertile cultivar Fujiminori. The donor- recipient method has been successfully used to transfer cytoplasmic male sterility (CMS) from indica variety Chinsurah Boro II into Japonica cultivars. Somatic hybrids were produced through protoplast fusion between rice and four wide species of *Oryza* but till now, no pronounced results have been achieved by using this technique.

3- Transformation:

Transgenic plants in rice were first produced by three groups. Toryama *et al.*, (1988), Zhong *et al.*, (1988), and Zhong and Wu (1988). Since then, transgenic plants have been produced in many laboratories in both indica and japonica rice carrying genes for herbicide tolerance, resistance to stem borer, virus resistance, resistance to fungal and bacterial pathogens, and other agronomic traits.

Goto *et al.* (1999) introduced the fourteen gene into rice. The transgenic plants showed increased accumulation of iron in the grain. Further studies are needed to determine the usefulness of engineered rice as a source of dietary iron. Several laboratories have produced transgenic rice, mainly through protoplast- mediated DNA transformation but also via microprojectile bombardment. Cheng *et al.* (1998) produced transgenic rice plants through *Agrobacterium* mediated transformation.

Tu *et al.* (2000) evaluated transgenic elite commercial hybrid rice expressing the *Bacillus thuringiensis* (Bt) genes cry IA (b) and cry IA (c) under field conditions. The transgenic plants showed a high level of protection to both leaf folder and yellow

stem borer. More recently, transgenic rice "golden rice" with the provitamin was produced. A (B- Carotene) biosynthetic pathway engineered into the rice endosperm.

Transgenic rice with Bt gene conferring resistance to stem borers (Alam *et al.* 1998, 1999; Cheng *et al.*, 1998; Datta *et al.*, 1998), bacterial blight resistance (Tu *et al.*, 2000), sheath blight resistance (Lin *et al.*, 1995; Datta *et al.*, 1999, Datta 2000 a, b), blast resistance (Nishizawa *et al.*, 1999) and stripe virus resistance (Hayakawa *et al.*, 1992) have already been developed. Transgenic resistant plants would produce more yield while using pesticide applications worldwide are now estimated to cost approximately,

\$ 8.1 billion per annum and Japan tops the list of pesticide users.

Methods of transformation that have been used are as follows; microinjection, macroinjection, macroinjection laser beam techniques, pollen tube pathway, DNA inhibition by dry seed and cell/tissue electroporation (Potrykus, 1991). However, the most reproducible results have been obtained from the protoplast, biolistic, and *Agrobacterium* methods (Datta, 2002).

4- Molecular markers:

In recent years, molecular markers have become attractive in varietal improvement, and could be used in the development of detailed genetic and physical chromosome maps among the plant systems could be applied in improving the efficiency of conventional plant breeding by carrying out indirect selection through molecular markers linked to the traits of interest-both simple and quantitative traits loci. QTL- markers are not influenced by the environment and can be scored at all stages of plant growth. Molecular markers can be utilized in germplasm characterization, genetic diagnostics, characterization of transformants, study of genome organization, phylogenetic analysis etc. Moreover, Singh *et al.* (1998) noted that molecular markers attracted breeders on the introgression of single or minor genes from wild species or cultivated donor lines into otherwise superior varieties to improve yield and tolerance for drought and heat.

Molecular markers have been classified in two main categories, the first are biochemical marker techniques which include protein and isozyme markers. The second are the DNA marker techniques which include RFLP, RAPD, AFLP, minisatellites and microsatellites simple tandem repeats (STR), or simple sequence repeats (SSR) or PCR -based markers.(Gupta *et al.*, 1999).

A molecular genetic map of rice containing 135 markers based on RFLPs was developed at Cornell University in collaboration with IRRI (McCouch *et al.*, 1988). Different maps were developed according to the molecular marker. The availability of

comprehensive molecular map in rice has opened new overuse to tag genes governing agronomic traits with molecular marker.

This has led to major advances in marker assisted selection and pyramiding of useful genes. McCouch *et al.*, (1991) and Yu *et al.*, (1991) were the first to tag genes for bacterial blight and blast resistance with molecular markers (Khush and Brar, 1998).

Some examples include genes for blast resistance, P.I., (t), Pita, Pi 5t , P, 7 (t), Pi 9 (t), Pi 10 (t), Pi 11 (t), and Pib; bacterial blight resistance – Xa I, Xa 2, Xa 3, Xa 4 , Xa 5 , Xa 10, Xa 13 and Xa 21; brown plant hopper resistance-Bph1 and Bph 10 striped virus resistance (Stvb1), thermosensitive male sterility (tms 2, tms 3), and photoperiod sensitivity (Se 1, Se 3). The major development involves the use of molecular markers to indentify Qtls from unadapted germplasm or wild species that can enhance the grain yield of rice. Two yield –enhanceing loci (Y/d 1, y/d 2) located on chromosome 1 and 2 of O-rufipogon have been identified (Xiao *et al.*, 1996).

The molecular markers assist in gene pyramiding if these markers are tightly linked with target genes. PCR-based DNA markers have facilitated the deployment of MAS in rice breeding. In MAS, individuals carrying target genes are selected in a segregating population based on linked markers rather than on their phenotype. The population can be screened at any stage of growth and in various environments. MAS increases the efficiency of a breeding program by selecting for markers linked to target traits or QTLS.

Singh *et al.* (2001) used MAS to pyramid genes for bacterial blight resistance into a high yielding cultivar RP 106, that is susceptible to bacterial blight. MAS has been useful in pyramiding recessive genes for resistance to bacterial blight such as Xa 5 and Xa 13 with a dominant gene Xa 21, which confers resistance to many races and thus masks the resistance conferred by recessive genes.

MAS was also employed to pyramid genes for resistance to blast.

Structural and functional genomics:

Major advances have been made in sequencing the rice genome under the International Rice Genome Sequencing Project (IRGSP) in Tsukuba, Japan which began in 1998. This collaborative effort among 15 laboratories in 10 countries aims to produce publicly available sequence data for the complete genome of rice. Each country has been assigned responsibility to sequence a specific rice chromosome (Number 1- Japan, Korea, 2- UK; 3- USA, 4- China, 5- Taiwan; 6,7,8 – Japan; 9- Thailand, Canada; 10-USA; 11-USA, India; 12- France, Brazil).

China nowadays started alone to find out the functional activity of the structural genes.

Application of molecular markers in rice breeding in Egypt :

Application of molecular markers such as isozyme and random amplified polymorphic DNA (RAPD) to study genetic diversity and germplasm management is a routine practice in RRTC, TCL. The work has been done to study the differences between different genotypes especially those tolerant to salinity and drought.

1- Protein variations between some rice varieties:

This technique was used as useful for discriminating among different cultivars; Japonica, Indica/ Japonica and Indica differed in their background and their performance either salinity and drought.

Eleven rice genotypes (local and exotic) were subjected to gel electrophoresis to differentiate between them according to banding pattern. Five bands were the main bands and presented in all varieties with very strong intensity. Agami M.1 and Nabatat Asmar have some bands, fortunately they are Japonica and tolerant to salinity. Moreover, varieties like Giza 178, Gaori, GZ 5310-20-3 and IR 65829-2B-2R-P have some banding patterns, these varieties are similar in their performance to salinity. While IET 1444, Giza 178, Wab 326-B-B-15 and IRGA 317-56-1F-1-2 have the same bands, these varieties are drought tolerant.

2- Isozyme markers:

Isozymes are the multiple molecular forms of an enzyme having the same catalytic specificity but differing in kinetic properties.

Isozyme markers have been employed for constructing the genetic linkage maps as well as studying the differences in isozyme patterns between parental materials and hybrids.

Isozyme variation between Egyptian varieties :

Both Esterase and Peroxidase isozymes were used to differentiate between nine varieties i.e., Giza 177, Giza 178, Giza 181, Giza 182, sakha 101, Sakha 102, sakha 103, Sakha 104 and Egyptian Yasmine. The two salt tolerant ones Giza 178 and sakha 104 have the same bands, may be these bands related to tolerance to salinity.

Prospective:

- 1- Wide application of MAS in breeding programs.
- 2- Transformation of novel genes for abiotic stresses.
- 3- Developing of ideotype through haploid breeding and developing anther culture derived lines having salt and drought tolerance as well as blast resistance.
- 4- Open new areas for new projects with Mediterranean region countries takima into consideration blast, salinity as well as grain quality.

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التكنولوجيا الحيوية ودورها فى تحسين الأرز فى مصر : التطبيقات والمشاكل والرؤية المستقبلية

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تعتبر الزراعة المستدامة هى التحدى الرئيسى فى المستقبل، فى عام ٢٠٢٥ يجب أن يتضاعف الإنتاج للحصول على كميات ونوعيات كافية من الغذاء وذلك لمجابهة الزيادة السكانية والإستهلاك المتزايد. ومن أهم أهداف مربي الأرز فى مصر هو تعظيم الإنتاجية من وحدة المساحة ولقد استطاع مربوا الأرز استنباط أصناف تعطى أعلى إنتاجية. وهناك استراتيجيات واضحة لزيادة الإنتاج من وحدة المساحة وهى استخدام تكنولوجيا الأرز الهجين والاهتمام بإنتاجية الأراضى الملحية وكذا استخدام التكنولوجيا الجديدة.

لقد أصبحت التكنولوجيا الحيوي ذات أهمية خاصة فى تحسين الأصناف من ناحية المقاومة للأمراض والحشرات وتحمل الإجهادات الغير حيوية مثل الملوحة وغيرها.

وهناك عديد من التكنولوجيا الحديثة التى تستخدم فى التحسين الوراثى للأصناف مثل زراعة الأنسجة والتربية الأحادية وزراعة الأجنة والتحليل الجينومى ونقل الجينات وأهرمة الجينات ودراسة التراكيب الجينومية من ناحية التركيب والوظيفة.

استخدمت فى مصر طريقة التربية الأحادية مثل زراعة المتوك ومن خلالها أمكن استنباط العديد من السلالات . التى بلغ محصولها حوالى ١٠ طن / هكتار . وأمكن أيضا استنباط سلالات عالية التحمل للملوحة وذات أميلوز منخفض وذات مقاومة ذاتية للحشائش. أمكن استخدام طرق المعلامات الجزيئية منها البروتين ومثابهاات الأنزيمات للترقية بين الأصناف.