INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–0568/201x/00–0–000–000
DOI: 10.17957/IJAB/15.0798
http://www.fspublishers.org

Full Length Article

Development of Nutrient-rich and Blast-resistant Rice Cultivars through Tissue Culture and Monogenic Lines

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Abstract

The breeding of rice varieties with higher levels of vitamins and the ability to resist disease has become one of the most important goals in plant breeding programmes. In this experiment, 24 F_{1s} were produced through Line × Tester and evaluated with their parents for genotypic variation. Both general combining ability (GCA) variances were highly significant for all twelve characters studied in F_1 generations. The most promising hybrid combinations were IRBL3-CP4 × Eg-N-9, IRBL5-M × Sakha101, Sakha106 × Al-Ahsa Type1, and Sakha106 × Al-Ahsa Type2, and these could be involved in breeding programmes. The protein level and nutrient elements; zinc (Zn) and iron (Fe) revealed that the two Saudi varieties; Al-Ahsa Type1, Al-Ahsa Type2 and Egyptian variety, Eg-N-7, had the highest value of protein, Zn and Fe. Therefore, ten genotypes out of fifteen were cultured *in vitro* to induce somaclonal variation. Seven genotypes were successfully induced callus and somaclonal were collected and evaluated for quantitative trait such as; duration, leaf blast, plant height, number of panicles/plant, 1000-grain weight, number of grains/panicle, grain yield and number of sterile grains/panicle and compared with the parents. The differences between the lines were remarkably slight compared with their parents: these lines could be included in breeding programmes as a genetic resource. Regarding the identification of blast- resistant genes, marker assisted-breeding Al-Ahsa Type1 and Al-Ahsa Type2 varieties had different blast-resistant genes. The finding of the study may help development of nutrient-rich and blast-resistant varieties of rice. © 2018 Friends Science Publishers

Keywords: Blast (*Magnaporthe grisea*); Dehusked mature seeds; Nutrition; Rice (*Oryza sativa* L.); Simple sequence repeat; Somaclonal variation
Abbreviations: 2,4-D − 2,4-Dichlorophenoxyacetic acid; BA− Benzyl Adenine; MS – Murashige and Skoog Medium; NAA − 2-Naphthalene Acetic Acid; SC- Somaclonal; SSR-Simple Sequence Repeat

Introduction

Rice production is one of the most important economic activities around the world, especially as a cash crop (GRiSP, 2013). It is the main human food source globally (Cantrell and Reeves, 2002). Rice supplies more calories to people's diet than other main crops, such as wheat, maize and potato. The calories from rice are particularly important in Asia and Africa, where it forms about 50-80% of daily caloric consumption (Seck *et al.*, 2012; Futakuchi *et al.*, 2013). Rice delivers 23% of international human *per capita* energy and 16% of per *capita* protein (Ye *et al.*, 2000). However, the diet of the urban and rural poor is often rice only, which lacks of alternative food in the form of enriched foods and a diversified diet may lead to loss to the body of thiamine and vitamins A and B. This in turn lead to the danger of blindness in children in the developing world, and also to Beriberi, a disease resulting from the consumption of white rice (Zapata-Arias, 2003).

Breeding crops with higher levels of important elements, such as vitamins, minerals, protein and healthier fats, is the most practical way to improve public health. For that reason, several studies have been conducted with the aim of combatting widespread vitamin A deficiency by incorporating lines of 'golden' rice and iron-rich rice to combat iron deficiency (Perez *et al.*, 1987; Beyer and Al-Babilis, 2002). Golden rice (GR) was rice genetically engineered to be rich in β-carotene as a source of vitamin A (Ye *et al.*, 2000). Tang *et al.* (2012) reported that the β-carotene in GR is as effective as pure β-carotene in oil and better than that in spinach as a source of vitamin A for children. However, the improved traits in terms of nutrition value need to have additional features, such as resistance to blast.

To cite this paper: Hassan, S.M.A.Z., O.A. Almaghrabi, M.M. El-Malky, E.M.R. Metwali and H.Z. Rihan, 201x. Development of nutrient-rich and blast-resistant rice cultivars through tissue culture and monogenic lines. *Int. J. Agric. Biol*., 00: 000–000

Rice blast is a devastating disease that occurs all over the world (Nguyen *et al.*, 2015; El-Malky *et al.*, 2017). The blast causes a high amount of damage to rice and yield loss estimated about 30% - 100% worldwide, depending on the agro-system. Fan *et al.* (2017) stated that the breeding of rice that is resistant to diseases such as blast is the most effective strategy of disease control. One of the essential strategies for managing blast diseases is utilization of multiple R gene (Sharma *et al.*, 2012). Conventional breeding was used through hybridization between nutrient rich varieties and monogenic resistant lines. with different resistance genes for blast (Fukuta *et al.*, 2004; Telebanco-Yanoria *et al.*, 2008; Takehisa *et al.*, 2009; Koide *et al.*, 2011; El-Malky *et al.*, 2016).

Traditional breeding techniques have failed to respond efficiently to demand and to deliver fast and efficient solutions for crop improvement. The 'green revolution' was dependent to a large extent on plant breeding techniques to produce plants with desirable traits: higher yield, high nutritional value and resistance to diseases. One of the most important new breeding techniques used in biotechnology is *in vitro* culture (Dalla Costa *et al.*, 2017). Techniques of tissue culture and somatic hybridization; the culturing of small explants, such as hypocotyl, cotyledon, mature or immature embryo and the small growing tips of plants, such as shoot apex, in the laboratory on specific nutrient media, offer vast potential for manipulation of plants *in vitro* for the production of new varieties on a large-scale (Jain, 2002). Embryo culture has been applied to rescue hybrid plants from wide crosses, but this has usually failed to produce mature viable seeds. In these situations, the immature embryo tissue could be removed and cultured in artificial media in the laboratory, to produce hybrid plants. Embryo culture allows the breeder to make wide crosses effectively with a higher number of related species of wild plants, and to gain access to a wide range of genes that could be utilized for the genetic improvement of crop plants. Embryo culture and wide crosses are valuable tools, especially for the transfer of disease resistant genes from wild relatives into crop plants (Suslow *et al.*, 2002). Optimization of tissue culture protocols for monogenic lines will help to create desired plant types that are better suited for cultivation and will improve the nutritional value of the rice seeds, as well as improving disease resistance to blast.

Various molecular markers, such as inter-simple sequence repeat (ISSR), microsatellite or simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) have been used for DNA fingerprinting, and to test plants for the inheritance of many different genes, germplasm characterization, measurement of genetic variation, analysis of genetic diversity in populations and genetic stability in several breeding programmes (Rani and Raina, 2000; Shao *et al.*, 2010). Over the years, advances in the methodology of molecular genetics have led to widespread use of co-dominant molecular markers, especially Simple Sequence Repeats (Vieira *et al.*, 2016). SSR distribution was found to be highly non-random and to vary a great deal in different regions of the genes of *Arabidopsis thaliana* and rice (Lawson and Zhang, 2006). Microsatellite markers or SSR indicate the distinction of individual heterozygotes from homozygotes; help in the identification of multiple alleles present in populations; determine the genetic reliability, population structure, genetic mapping, evolutionary processes, synthesis of easily interpretable results having high reproducibility, and can be utilized to expedite the introgression of resistance genes into novel cultivars (Alba *et al.*, 2009; Awan *et al.*, 2017). There is lack of information about the development of nutrient-rich and blast resistance varieties of rice. This study was, therefore, conducted to evaluate a wide range for rice genotype for selecting nutrient-rich and blast resistance varieties of rice. Somaclonal variation techniques were used for improving the grain nutrient contents and blast resistant in the tested rice genotype. Identification of blast resistant genes was also one of the objectives of the study.

Material and Methods

This study was conducted at the Biotechnology laboratory of the King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia and the experimental farm of Rice Research and Training Center (RRTC), Sakha, Kafr El-Sheikh, Egypt, during the years 2015, 2016 and 2017. The plant materials used were 15 genotypes of rice (*Oryza sativa* L.) including two Saudi genotypes; Al-Ahsa Type1 and Al-Ahsa Type 2, obtained from the Agriculture Research Center, Al-Ahsa, Kingdom of Saudi Arabia; ten Egyptian genotypes of varying nutrient values procured from the Rice Research and Training Centre (RRTC), Egypt; and three monogenic lines, received from the International Rice Research Institute (IRRI), Los Baños, the Philippines (Table 1).

Field Experiment

In the 2015 season, progress of evaluated and selection of the fifteen genotypes under study were implemented by estimation of nutrient elements and protein percentage. Of the fifteen parents' genotypes, only ten genotypes were selected and involved in the hybridization in the 2016 season, using design Line × Tester mating to produce 24 crosses. Six genotypes namely; IRBLKS-S, IRBL3-CP4, IRBL5-M (monogenic lines), Eg-N-1, Eg-N-2 and Sakha106 were used as "Lines", while the genotypes Eg-N-9, AL-Asha Type1, Al-Ahsa Type2 and Sakha101 were used as "Testers". IRBLKS-S, IRBL3-CP4, IRBL5-M, Eg-N-1, Eg-N-2, Sakha106, Eg-N-9, AL-Asha Type1, Al-Ahsa Type2 . A hot water method for emasculation was utilized for the hybridization (Jodon, 1938; Butany, 1961). In the 2017 season, F_1 seeds and their parents were grown in plots. Each plot contained three rows and each row contained 25 plants, grown at a spacing of 20 × 20 cm using randomized complete block design (RCBD) with three replications.

Table 1: Genotypes, parentage pedigree, nutrient level, origin and blast reaction for 15 different genotypes of rice

No.	Genotypes	Parentage	Origin	Nutrient level	Blast resistance
1	Eg-N-1	Mizuho/Zenith//Black Rice	Egypt	High	R*
2	Eg-N-2	Pure line selection	Egypt	High	R
3	Eg-N-9	Pure line selection	Egypt	High	R
4	Al-Ahsa Type1	Exotic (Japan)	Saudi Arabia	Low	S**
5	Al-Ahsa Type2	Exotic (Japan)	Saudi Arabia	Low	S
6	IRBLKS	Lijiangxintuanheigu/SHIN2	IRRI	Low	R
7	IRBL3-CP4	Lijiangxintuanheigu/C104PKT	IRRI	Low	R
8	IRBL5-M	Lijiangxintuanheigu/RIL 249	IRRI	Low	R
9	Sakha102	Gz4096-7-1 / Giza177	Egypt	Low	R
10	Sakha101	(Giza 176/Milyang 79)	Egypt	Low	S
11	Sp-70	Sakha101/HR5824//Sakha101	Egypt	Low	R
12	Giza178	Giza175 / Milyange49	Egypt	Low	R
13	Saka105	Gz5581 / Gz4316	Egypt	Low	R
14	Sakha106	Giza177 / Hexi30	Egypt	Low	R
15	Giza177	(Giza 171 / Yu mji No.1 // piNo.4)	Egypt	Low	R

* R= Resistant ** S= Susceptible IRRI= International Rice Research Institute

Four agronomic traits were recorded: number of tillers per plant, 1000-grain weight, number of spikelet sterility/plant and grain yield per plant.

Estimation of Micronutrient and Protein Percentage

Milled grain samples for fifteen genotypes (Table 1) were collected in the 2015 season and oven dried at 70°C until the weight was constant. Sub-samples from grain were taken and then placed in bags and oven dried at 70°C for 48 h. Dried samples were ground to powder and digested by wet oxidation procedures (Johnson and Ulirch, 1959). Zn and Fe elements were estimated following HarvestPlus protocol (2006), using an atomic absorption spectrophotometer (AAS200). Protein contents in milled rice grain were estimated, following procedures described by Johari *et al.* (2000).

Tissue Culture Experiment

Callus induction and maintenance: A total of 12 de-husked mature seeds for each of the ten selected genotypes were sterilized by submerging in 20-30% Sodium Hypochlorite and kept on shaker for 5 min, then washed three times with sterilized double distilled water under aseptic conditions in a laminar airflow hood. Four immature seeds of each genotype were cultured in petri dishes and a completely randomized design with three replications was set. Each Petri dish contained 15 mL of agar solidified basal MS medium, (Murashige and Skoog, 1962), supplemented with 100 mg L^{-1} Myo-inositol, 1.0 mg L^{-1} thiamine-HCl, 3.0 mg L^{-1} 2,4-D, 3% sucrose. The pH was adjusted to 5.7 prior to autoclaving of the medium at 121°C and 15 psi for 20 min. The cultures were incubated in darkness at 25+1°C for 4 weeks to encourage callus initiation and induction. After four weeks, the frequencies of callus induction and the mean value of callus fresh weight were recorded.

Somaclonal Variation

Healthy friable calluses were chosen to obtain plant regeneration. Calluses were cultured in sterilized tissue culture jars (370 mL) consisting of MS medium (M1) as described before, but plant growth regulator 2,4-D was replaced with another hormone, 2.0 mg L^{-1} 6-Benzile Adenine (BA), according to El-Malky (1997). All cultures were incubated in a growth chamber according to Metwali *et al.* (2015). After 8 weeks the calluses regenerated into normal looking rice plantlets and were transferred to greenhouse for adaptation. Regenerated SC0 plants were cultured in small pots for one week and then transplanted to larger pots to grow until maturity stage. Seeds of each panicle were threshed separately to produce a line in the next season. In 2017, each line was cultivated in a separate row with its parent. 66 lines, derived from seven parents, were cultivated as individual plants for comparison with their parents. The vegetative characters included: duration, leaf blast, plant height (cm), number of panicles/plant, 1000-grain weight (g), number of grains/panicle, grain yield (t/ha) and number of sterile grains/panicle was measured on the plants of the current season (SC1 plants).

Molecular Marker Analysis

A total of fifteen rice genotypes were employed in the study to identify the blast resistance genes by using 15 specific primers purchased from Sangon Company, China**.** Molecular analysis was conducted at the EPCRS Centre (Certified according to ISO 9001, ISO 14001 and OHSAS 18001), Kafr El-Sheikh University, Egypt. DNA was isolated from the fifteen rice accessions according to Maixner *et al.* (1995). Selected symptomatic leaves were washed with clear water. 4 to 10 midribs were cut with a disposable razor blade and 1.0 g of midribs was dispensed in an ELISA sachet and 3.0 mL CTAB extraction buffer. Then the midribs were squashed under cooling 4ºC. 1.5-2.0 mL of midrib juice was transferred to 2 mL tube and kept in

a water bath at 65°C for 15 min. This was followed by centrifugation at 3000 for 5 min. One mL of supernatant was collected and transferred to an Eppendorf tube. 1 mL Chloroform-Isoamylic alcohol was then added, and mixed by inverting the tubes several times to obtain an emulsion. The emulsion was then kept in the Centrifuge for 5 min at 14000 ×*g*. Aqueous phase was collected, transferred to new tubes and 540 μL Isopropanol was supplied, left at -20°C for 30 min, then centrifuged for 20 min at 14000 ×*g*. After centrifugation, ethanol was removed without disturbing the small nucleic acid pellet. The pellet was washed with 1 mL ethanol 70%, followed by centrifuge for 10 min at 14000 ×*g*. Ethanol was removed and the pellet was dried in speed-VAC*5 min. The dried pellet was re-suspended with 60-100 μL TE 1X (Tris 10 m*M* EDTA 1 m*M* PH8) and nucleic acid stored at -20°C.

Polymerase Chain Reaction Assay

The reaction mixture (25 μL) consisted of: 12.5 μL of 2x master mix ready to use (0.1U/µL *Taq* Polymerase, 500 µM dNTP, 20 m*M* Tris-HCl (pH8.3), 100 m*M* KCl, 3 m*M* MgCl2 and Stabilizer and enhancer) + 10 Pmol of each primer (1.0 μL) + 1.0 μL of DNA (50 ng) + 9.5 μL PCR grade water. Amplification was performed in a Thermocycler (Bio-Rad, C - 1000) as follows: (1). Initial denaturation at 94°C for 5 min. (2) Denaturation at 94°C for 30 sec. (3) Primers annealing temperature differing according to Tm of each gene for 1 min. (4) Extension at 72°C for 1 min. (5) Steps 2, 3, 4 are repeated 40 cycles. (6) A final extension at 72°C was given for 10 min. After PCR, the amplified products were analyzed on 1.5% agarose gel containing ethidium bromide to a final concentration of 0.5 µg mL^{-1} as follows: electrophoresis grade agarose (0.9 g) was prepared in 60 mL TAE 1x buffer in a sterile flask. It was heated in a microwave to dissolve all granules with agitation, and allowed to cool at 70°C; and then 0.5 µg mL^{-1} ethidium bromide was added and mixed thoroughly. The warm agarose was poured directly into the gel casting apparatus with desired comb in apposition and left at room temperature for polymerization. The comb was then removed, and the electrophoresis tank was filled with TAE (1x) buffer. Ten μL of amplified product was loaded into the well and run along with 1 Kb plus DNA ladder (Intron Biotechnology Company, Korea) in a 1x TAE electrophoresis buffer at 5 volts/cm2 for 45 min. At the end of the electrophoresis, the gels was transferred to a UV cabinet. The gels were then photographed and analyzed, using BioDoc Analysis software (Biometra, Germany).

Statistical Analysis

In field and tissue culture experiments, analysis of variance (ANOVA) was applied to statistical analysis of experimental data, using statistical MSTATC (Snedecor, 1956). Differences between individual means were estimated, using Duncan's multiple range (Duncan, 1955; Singh and Chaudhary, 1985). All values are reported as means ± standard deviation.

Results

Field Experiment

Analysis of variance showed highly significant differences for all studied characters, indicating overall differences among those genotypes. The parents, parent *vs.* crosses, line, tester and line × tester were shown to be highly significant for all characters under study, indicating a wide range of differences among parents and crosses, and between parent vs crosses and, on the other hand, among line and tester and between line × tester (Table 2). The mean square among genotypes was highly significant for all studied traits, indicating that rice genotypes differed in their genetic potential for such traits. Parents vs crosses were found to be highly significant for all traits (Table 3). Lines variance was highly significant for 1000 grain- weight and grain yield/plant. The interaction of line × tester was found to be highly significant for all studied traits. All lines, tester and line × tester, except Al-Asha Type (1), Al-Asha Type (2) and Sakha 101 varieties, exhibited high resistance to blast. GCA was found to differ significantly from zero in most cases. High positive values of GCA effects would be of interest in most traits under investigation (number of tillers/plant, 1000 grain weight (g) and grain yield/plant, while the negative value was desirable in number of sterile spikelets per panicle. IRBLKS-S, Sakha106, Eg-N-9 and Sakha101 were highly significantly positive for grain yield per plant, and proved to be good donors or combiners for these traits (Table 4).

Estimation of Micronutrient and Protein Percentage

The content of Zn and Fe and protein in milled rice of 15 rice genotypes are shown in Fig. 1. The results showed that the highest values of Zn and Fe nutrient elements were estimated in the genotypes, Eg-N -1, Eg-N -2, Eg-N -9 except Sakha105. On the other hand, protein concentrations in milled rice showed wide ranges of values - from 4.2 to 11.8%. Three genotypes Eg-N-1, Eg-N-2 and Eg-N-9 recorded the highest value of more than 10% protein in their milled grains. The lowest value of protein 4.2, 4.3 and 4.5 (%) was observed in the three monogenic lines, IRBLKS-S, IRBL3-CP4 and IRBL5-M, respectively. The genotypes Eg-N -1, Eg-N -2, Eg-N -9 were generally rich in protein % and nutrient and could be used to improve the nutritional value of rice, using either conventional or modern biotechnology methods.

Callus Induction and Maintenance

Callus initiation began with enlargement of the embryo area of the seed, and the radical started to grow until it reached 2-3 cm long. Radical growth stopped after 5-7 days and callus started to grow (Fig. 2).

Table 2: Analysis of variance and mean square of line x testers analysis for the studied traits

Source	df	No. of tillers/plant	1000-grain weight	No. of spikelet sterile/plant	Grain yield/ plant
Replication	2	0.38	0.22	4.59	0.29
Treatment	33	47.91**	60.79**	412.26**	199.96**
Parents	9	16.16**	15.15**	501.65**	295.09**
Parents vs. Crosses	1	270.65**	24.66**	302.22**	193.28**
Crosses	23	50.65**	80.22**	377.76**	163.03**
Lines	5	82.69**	80.83**	439.16**	173.53**
Testers	3	114.72**	156.23**	1531.04**	692.50**
Lines x Testers	15	27.15**	64.81**	126.64**	53.64**
Error	66	2.92	0.14	5.25	1.65

* and ** Significant at 0.05 and 0.01 levels respectively

Table 3: Mean performance of parents and their F_1 hybrid for studied traits

No.	Genotypes	No. of tiller / plant	1000-grain weight	No. of sterile spikelet/plant	Grain yield/ plant
1	IRBLKS-S	24.00	23.53	32.67	34.47
2	IRBL3-CP4	20.67	26.30	24.67	32.23
3	IRBL5-M	21.33	23.37	28.33	38.37
4	Eg-N-1	25.00	24.50	14.67	37.70
5	Eg-N-2	23.67	25.50	10.00	33.30
6	Sakha106	26.67	28.83	7.33	55.13
7	Eg-N-9	26.67	24.33	16.33	34.93
8	AL-Asha Type1	26.67	21.50	39.33	26.20
9	Al-Ahsa Type2	26.67	23.17	41.33	24.83
10	Sakha101	26.67	27.80	7.00	52.57
11	IRBLKS-S X Eg-N-9	30.00	25.37	20.67	46.17
12	IRBLKS-S X Al-Ahsa Type1	28.00	23.47	32.33	34.63
13	IRBLKS-S X Al-Ahsa Type2	26.67	24.27	22.33	35.40
14	IRBLKS-S X Sakha101	33.33	26.40	8.33	57.00
15	IRBL3-CP4 X Eg-N-9	24.67	23.40	14.00	38.07
16	IRBL3-CP4 X Al-Ahsa Type1	31.67	22.07	37.00	31.63
17	IRBL3-CP4 X Al-Ahsa Type2	31.00	23.33	36.67	30.47
18	IRBL3-CP4 X Sakha101	32.33	27.27	8.33	50.93
19	IRBL5-M X Eg-N-9	26.33	23.30	13.00	36.67
20	IRBL5-M X Al-Ahsa Type1	23.67	24.23	34.00	32.27
21	IRBL5-M X Al-Ahsa Type2	27.33	2.33	35.00	34.60
22	IRBL5-M X Sakha101	36.00	28.47	10.33	46.53
23	Eg-N-1 X Ng-N-9	25.00	22.17	9.67	37.93
24	Eg-N-1 X Al-Ahsa Type1	23.33	21.20	25.67	30.33
25	Eg-N-1 X Al-Ahsa Type2	26.33	21.43	19.67	34.37
26	Eg-N-1 X Sakha101	28.33	27.07	8.67	46.03
27	Eg-N-2 X Eg-N-9	21.33	23.40	12.67	38.33
28	Eg-N-2 X Al-Ahsa Type1	22.33	21.80	28.33	34.33
29	Eg-N-2 X Al-Ahsa Type2	23.33	22.13	29.67	35.43
30	Eg-N-2 X Sakha101	32.67	28.03	4.67	43.97
31	Sakha106 X Eg-N-9	32.67	27.20	12.00	49.97
32	Sakha106 X Al-Ahsa Type1	33.33	27.93	6.00	49.00
33	Sakha106 X Al-Ahsa Type2	31.33	27.83	6.00	40.50
34	Sakha106 X Sakha101	30.00	27.20	6.33	45.30
L.S.D 0.05		2.76	0.61	4.64	2.08
L.S.D 0.01		3.65	0.81	6.15	2.75

Subcultures were applied every 21-28 days for ten genotypes: IRBLKS-S, IRBL3-CP4, IRBL5-M, Eg-N-1, Eg-N-2, Eg-N-9, Al-Ahsa Type1, Al-Ahsa Type2, Sakha101 and Sakha106. The results indicated that most of the genotypes succeeded in inducing callus, except for three monogenic lines; IRBLKS-S, IRBL3-CP4 and IRBL5-M. Callus induction percentage ranged from 75–100% (Table 5). The genotypes Eg-N-1, Eg-N-9, sakha101 and Sakha106 recorded the highest value (100%), followed by Al-Ahsa Type1, Al-Ahsa Type2 (85%) for callus induction. The lowest value for callus induction (75%) was obtained from the Eg-N-2 genotype. For initial callus fresh weight and callus fresh weight after 30 days, the genotypes Al-Ahsa Type1 and Al-Ahsa Type2 showed the highest values (0.698 and 0.765; 1.126 and 1.321), respectively. Excellent friable and healthy calluses were used to culture on regeneration medium. Successful callus regeneration was transferred for adaptation.

Somaclonal Variation

Seeds for seven somaclonals, obtained from *in vitro* tissue culture, were collected (SC0) and adapted to complete their growth in soil to produce SC1.

Table 4: Estimation of general combining ability (GCA) effect for studied characters

Genotypes	No. of tillers/plant	1000-grain weight	No. sterile spikelet/panicle	Grain yield/ plant
IRBLKS-S	1.13	1.07**	2.53*	3.31**
IRBL3-CP4	1.54*	0.21	5.61*	-2.22**
IRBL5-M	-0.04	-4.22**	4.69*	-2.48**
Eg-N-1	-2.63*	-0.84**	-2.47*	-2.83**
Eg-N-2	-3.46**	0.04	0.44	-1.98*
Sakha106	3.46**	3.74**	-10.81**	6.20**
S.E (gca)	0.49	0.11	0.83	0.37
S.E (gl- gj)	0.70	0.15	1.17	0.52
LSD 0.05	1.27	0.28	2.13	0.95
LSD 0.01	2.81	0.62	4.73	2.11
Eg-N-9	-1.71*	0.33*	-4.72*	1.19*
Al-Ahsa Type1	-1.32*	-0.35*	8.83**	-4.63**
Al-Ahsa Type2	-0.71*	-3.58**	6.50**	-4.87**
Sakha101	3.74**	3.60**	-10.61**	8.30**
S.E (gca)	0.16	0.04	0.28	0.12
S.E (gl- gj)	0.57	0.13	0.96	0.43
LSD 0.05	0.52	0.12	0.88	0.39
LSD 0.01	3.33	0.73	5.59	2.50

* and ** Significant at 0.05 and 0.01 levels respectively
gca; general combining ability gi, gj; GCA effects of the ith and jth parents, respectively

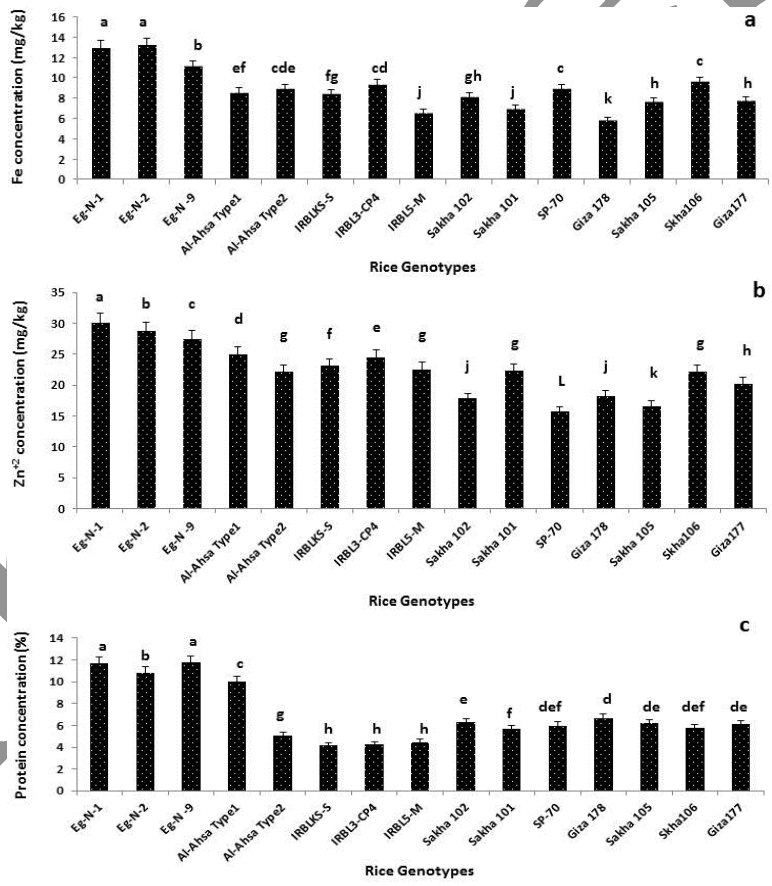

Fig. 1: Mean concentration of Fe and Zn nutrient and protein percentage in milled rice of 15 genotypes
Bars sharing the same letters represent no significant differences between means at $P \leq 0.05$ level

Different morphological characters were recorded and compared with the parents. A total of 66 plants were included in the SC1, derived from Eg-N-1 (12 plants), Eg-N-2 (6 plants), Eg-N-9 (8 plants), Sakha101 (6 plants), Sakha106 (9 plants), Al-Ahsa Type1 (14 plants) and Al-Ahsa Type2 (11 plants).

Table 5: Callus induction percentage, initial callus weight and callus fresh weight of 10 genotypes of rice (*Oryza sativa* L.)

No.	Genotypes	Callus induction%	Initial callus weight (g.)	Callus fresh weight (g.) after 30 days.
1	IRBLKS-S	-	-	-
2	IRBL3-CP4	-	-	-
3	IRBL5-M	-	-	-
4	Eg-N-1	100	0.432	0.9645
5	Eg-N-2	75	0.564	0.9321
6	Eg-N-9	100	0.475	0.9875
7	Al-Asha Type 1	85	0.765	1.321
8	Al-Asha Type 1	85	0.698	1.126
9	Sakha101	100	0.476	0.9764
10	Sakha106	100	0.488	0.8790
σ^2		107.143	0.048	0.057
STDEV		10.351	0.127	0.150
SE.		3.912	0.016	0.023
CV		11.234	22.817	14.620

-; no growth, σ^2; Variance, STDEV; Standard Deviation, SE; Standard Error, CV; Coefficient of Variation

SC1 family derived from Eg-N-1: data showed that all the lines were earlier than the parent and the lines 6, 8 and 11 were of short duration compared with the parent. For plant height, the lines 5, 10 and 11 were short statures one. As for yield character, the lines 7, 1, 6 and 9 gave the highest yield/plant (Table 6). The results of the SC1 family derived from Eg-N-2 indicated that three lines (4, 6 and 2) were of short duration, and gave 126.33, 127 and 128 days respectively. Concerning plant height, all the lines were of short stature, except line 1, which was higher than the parent. For grain yield/plant, the results revealed that all the lines were higher than the parent, except for line 5 (Table 6). While SC1 family derived from Eg-N-9, eight lines were studied under this population and the results indicated no significant difference between the lines and the parents for duration trait. For plant height, five lines gave 103.33, 105.33, 105.67, 106.33 and 106.67, respectively. For grain yield/ plant, the eight lines were higher than the parent and could be used as a donor in a breeding programme for this trait (Table 6). The lines were better than the parent in resistance to blast reaction in the SC1 family derived from Sakha101, and all the lines recorded a high value in grain yield (Table 6). The results for the SC1 family derived from Sakha106 displayed remarkably slight differences in from Sakha 106 in terms of duration and lines 9, 2 and 3 were a shorter one in in terms of duration 121.67, 122.33 and 123.33 days respectively, compared with the parent (127 days) (Table 6). These lines could be included in earliness breeding programmes. In the SC1 family derived from Al-Ahsa Type1, all the lines were shorter in duration trait compared with the parent. Also, for blast reaction, the lines were resistant and the plants of shorter stature. However, the data indicated that there were significant differences among the lines in grain yield/plant (Table 6). For the SC1 family derived from Al-Ahsa Type2, eleven lines produced from Al-Ahsa Type2, line 1 was earlier one in terms of duration (119 days), while the other lines were less than the parent. For blast reaction all the lines were resistant (Table 6). According to grain yield/plant, the line 5, 8, 4, 9 and 10 gave the high yield of 40.23, 40.13, 40.07, 39.47 and 39.30, respectively.

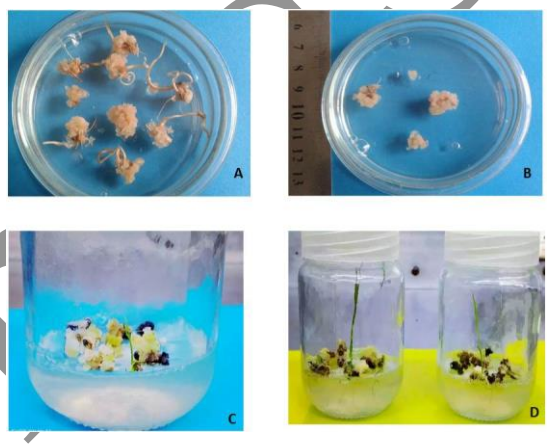

Fig. 2: A) Callus inducing emerging from the Rice embryo cultured on MS media with 3% (w/v) 2, 4-D (4 weeks); **B)** Maintenance of callus through subculture on MS media with 3% (w/v) 2, 4-D, subcultured every 5 weeks; **C)** Abnormal malformed green structures developed from callus growing on MS media supplemented with BA 2% (w/v) (6 weeks); **D)** Plant Regeneration (9 weeks)

Identification of Blast Resistance Genes

In this study, fifteen DNA markers were used for molecular analysis. These consisted of 5 SSR and 10 SNP.

RM1216 marker, which linked to the *Pish* gene, showed four alleles with fifteen rice genotypes: Eg-N-1, Eg-N-2, Eg-N-9, Al-Ahsa Type1, Al-Ahsa Type2, IRBLKS, IRBL3-CP4, IRBL5-M, Sakha102, Sakha101, Sp-70, Giza178, Sakha105, Sakha106 and Giza177. The results illustrated that the positive allele with molecular weight of 140 bp appeared with Eg-N-1, Eg-N-2, Eg-N-9, Al-Ahsa Type1, Al-Ahsa Type2, IRBL5-M and Giza178 genotypes and was absent in the other varieties. However, the Saudi Arabian and Egyptian nutrient genotypes carried the *Pish* genes, while the Egyptian non-nutrient genotypes did not carry this gene, except for the Giza178 genotype. The pedigree of the latter genotype was different, since it belongs to the Indica-Japonica type, whereas the other Egyptian genotypes belong to Japonica type.

Table 6: Vegetative and yield traits of somaclonal plants of SC1 generation derived from seven genotypes

Genotype	Duration	Leaf blast	Plant height	number of panicles/plant	1000 grain weight	number of grains/panicle	grain yield/plant	Number of sterile spikeltes/panicle
Somaclonal plants of SC1 generation derived from Eg-N-1genotype.								
Parent(Eg-N-1)	127.00	2.00	95.00	21.67	24.500	99.23	37.70	14.67
Line 1	125.33	1.00	92.33	22.00	24.700	112.43	39.23	14.00
Line 2	125.33	1.00	90.67	20.33	25.967	106.80	38.63	13.00
Line 3	121.00	1.00	90.00	23.00	24.667	104.83	37.97	8.00
Line 4	120.00	1.33	94.00	21.00	25.200	98.90	36.63	13.33
Line 5	122.00	1.00	88.67	18.67	23.800	100.90	38.23	6.67
Line 6	118.67	1.67	91.00	20.33	25.100	110.27	39.17	5.67
Line 7	122.00	1.33	93.00	23.33	24.267	101.03	40.40	9.00
Line 8	118.33	1.33	91.00	22.67	24.067	96.20	36.10	14.00
Line 9	120.00	1.33	89.33	16.67	23.133	108.43	39.17	9.00
Line 10	122.67	1.00	88.33	21.33	24.033	97.03	36.93	13.33
Line 11	118.67	1.33	88.33	20.33	25.667	104.77	38.40	5.33
Line 12	120.00	1.33	91.00	17.67	24.367	97.07	36.40	9.33
L.S.D 0.05	2.077	0.726	1.500	3.320	0.8779	3.593	1.323	4.159
Somaclonal plants of SC1 generation derived from Eg-N-2genotype.								
Parent(Eg-N-2)	131.00	1.667	85.00	16.67	24.80	118.00	37.40	8.67
Line 1	130.00	1.667	86.67	20.33	25.36	121.33	39.33	6.00
Line 2	128.00	1.667	83.00	18.67	24.70	125.00	39.90	7.33
Line 3	130.00	1.000	82.33	20.67	26.03	117.00	37.30	5.00
Line 4	126.33	1.000	83.00	17.00	24.96	122.67	40.23	4.67
Line 5	132.00	1.333	80.00	20.67	24.06	118.67	35.70	3.00
Line 6	127.00	1.000	82.67	21.00	24.76	125.33	39.50	4.33
L.S.D.0.05	1.624	0.7263	2.173	2.481	0.484	4.33	1.626	3.432
Somaclonal plants of SC1 generation derived from Eg-N-9genotype								
Parent(Eg-N-9)	129.67	2.000	111.00	21.00	23.73	167.00	31.00	5.60
Line 1	128.00	1.333	106.33	21.67	24.90	163.00	35.67	4.33
Line 2	129.00	1.000	108.67	21.67	24.90	190.00	35.33	5.67
Line 3	130.00	1.333	105.67	21.33	24.73	160.67	38.00	3.67
Line 4	127.00	1.000	103.33	22.00	24.83	156.33	35.33	3.33
Line 5	131.00	1.333	109.33	20.00	24.26	173.33	36.33	7.33
Line 6	128.00	1.000	110.00	22.00	23.73	177.67	34.00	4.67
Line 7	130.00	1.000	105.33	22.00	24.13	159.00	35.33	4.33
Line 8	128.67	1.333	106.67	21.00	25.03	172.33	36.67	3.67
L.S.D.0.05	2.136	0.666	3.858	2.393	0.4629	7.939	4.637	2.415
Somaclonal plants of SC1 generation derived from Saka101genotype.								
Parent(Sakha101)	145.00	6.67	95.3	21.67	26.53	131.00	42.10	15.00
Line 1	139.33	3.00	96.0	23.00	26.70	140.33	44.13	8.00
Line 2	141.67	2.67	96.0	21.67	27.03	141.33	46.17	8.67
Line 3	143.67	2.67	95.6	22.67	26.76	141.33	44.90	7.67
Line 4	139.00	2.33	95.0	22.67	26.76	137.67	43.67	11.67
Line 5	142.33	2.67	94.6	24.00	26.50	143.00	45.33	9.33
Line 6	140.67	2.00	94.6	23.67	27.00	141.00	44.43	11.33
L.S.D.0.05	3.275	1.087	1.960	2.680	0.455	5.791	2.204	4.075
Somaclonal plants of SC1 generation derived from Sakha106 genotype.								
Parent (Saka106)	127.00	1.667	106.67	19.00	27.76	137.33	40.03	4.67
Line 1	125.00	1.333	105.00	21.00	28.03	138.67	40.33	4.67
Line 2	122.33	1.000	106.00	21.67	27.80	140.00	40.00	4.00
Line 3	123.33	1.000	105.00	21.00	27.80	135.67	40.33	5.33
Line 4	125.33	1.000	105.00	20.67	27.66	137.33	39.67	3.67
Line 5	126.67	1.333	105.00	20.33	26.90	129.00	36.33	3.67
Line 6	124.33	1.667	105.33	18.00	27.06	138.00	41.67	5.33
Line 7	124.00	1.667	107.00	20.33	27.73	140.00	39.67	5.33
Line 8	124.67	1.667	105.67	17.67	27.10	135.33	41.00	5.00
Line 9	121.67	1.667	105.33	19.33	27.30	138.67	41.00	5.00
L.S.D.0.05	1.587	0.730	1.984	2.222	0.467	4.351	2.395	2.988
Somaclonal plants of SC1 generation derived from Al-Ahsa Type1 genotype								
Parent(Al-Ahsa Type1)	156.00	5.66	144.0	14.00	25.06	110.33	38.23	14.67
Line 1	149.33	2.33	139.0	16.67	26.16	120.33	40.73	9.33
Line 2	149.67	2.00	135.3	18.67	25.63	117.00	39.03	9.33
Line 3	153.33	2.00	140.0	20.00	26.50	122.67	38.37	5.67
Line 4	149.67	1.66	143.3	19.00	25.30	114.67	40.30	7.33
Line 5	147.33	1.33	136.0	19.33	24.23	124.33	41.23	5.33
Line 6	145.33	1.00	136.0	18.00	25.46	122.00	38.57	8.00
Line 7	153.00	1.33	134.6	19.67	24.06	110.00	40.33	5.00
Line 8	148.33	1.66	133.3	17.33	25.83	123.00	34.63	8.67
Line 9	151.33	2.00	141.3	17.67	24.73	128.33	37.90	9.00
Line 10	155.00	1.66	142.6	18.67	25.90	121.67	35.50	6.33
Line 11	148.67	1.33	135.3	19.67	24.83	109.33	39.50	6.67
Line 12	151.67	1.33	135.3	21.00	24.90	120.67	33.30	8.00
Line 13	146.67	1.66	133.0	18.33	25.10	123.33	42.13	9.33
Line 14	145.00	1.33	129.6	21.67	25.43	115.33	41.27	9.33
L.S.D.0.05	2.488	0.774	3.598	3.687	0.794	4.789	2.008	3.236

Table 6: Continued

Table 6: Continued

Somaclonal plants of SC1 generation derived from Al-Ahsa Type2 genotype.								
Parent(Al-Ahsa Type2)	156.7	5.66	143.0	14.67	23.50	96.67	35.87	31.33
Line 1	119.0	1.66	141.6	17.67	25.20	113.67	37.50	26.33
Line 2	150.7	2.66	140.6	18.33	25.23	118.00	37.23	30.67
Line 3	153.0	2.33	144.0	16.67	25.10	116.67	38.50	23.33
Line 4	155.0	2.33	138.3	18.33	25.53	114.00	40.07	23.33
Line 5	148.0	2.33	141.6	17.33	25.70	117.67	40.23	18.33
Line 6	152.3	1.66	138.0	18.00	25.83	119.33	35.57	18.33
Line 7	154.7	1.33	141.0	17.33	25.83	117.00	37.47	23.33
Line 8	144.0	1.33	140.3	18.33	25.76	121.00	40.13	22.67
Line 9	149.3	1.00	142.0	18.33	26.06	120.67	39.47	19.67
Line 10	148.0	1.66	138.3	18.33	25.83	122.67	39.30	17.67
Line 11	153.0	2.33	140.6	16.00	25.80	117.00	35.50	18.33
L.S.D.0.05	27.74	0.653	2.56	2.954	0.479	5.032	1.703	5.145

For RM44 marker, which linked with Pi33, showed positive band at size 161bp. This band was present in Eg-N-1, Eg-N-2, Sakha102, Sakha101, Sp-70, Sakha105, Sakha106 and Giza177, but absent from the other genotypes. The SSR marker RN3843 that was linked with the Pi39 gene presented a polymorphic banding pattern for the tested genotypes. Eight alleles were amplified with different molecular weights, and the positive band at 165 bp was present in all Egyptian genotypes, except Giza178, that is main these genotypes carrying Pi39 gene, while the other genotypes were not carrying this gene. Also, RM3330 linked to the Pi40 gene, and different polymorphic bands were observed. The results showed that the all the genotypes were carrying this gene, except for the varieties Sakha101 and Giza177, while, the results for SSR marker RM206, which was linked to the rice blast resistant gene *Pi38*, showed that the Al-Asha Type1, Al-Asha Type2, IRBLKS and Sp-70 carried *Pi38* with size banding 147bp.

The SNP marker K3957 is specific for the blast resistant gene *Pik*. Monogenic line IRBL5-M was used as positive control for the *Pik* resistant gene. All genotypes showed positive band with molecular weight 146 bp, which meant that all genotypes in this study carried *Pik* genes. The marker JJ817, was specific for rice blast resistance gene *Pi5* and monogenic lines were used as positive control of *Pi5*. The results showed that this gene was absent for all genotypes under study, except the monogenic lines, which had a size banding of 403 bp. Also, two SNP markers, JJ81-T3 and JJ113-T3, were specific for blast resistance genes *Pi3* and *Pii*, respectively. Monogenic lines IRBL3-CP4 and IRBLi-F5 were used as positive controls for *Pi3* and *Pii* respectively. The results showed that all the genotypes carried the resistance gene *Pi3* with size banding 314 bp. On the other hand, the JJ113-T3 showed positive with Al-Ahsa Type1, Al-Ahsa Type2, IRBL3-CP4, IRBL5-M, Sakha102, Sp-70, Giza178, Sakha105, Sakha106 and Giza177 with size banding 481bp, whereas it was absent from the other genotypes.

The specific dominant marker YL153/YL154 was used to identify rice blast resistant genes *Pita* and *Pita-2*. The genotypes Eg-N-2, Al-Ahsa Type1, Al-Ahsa Type2, Giza178, Sakha105 and Sakha106 recorded as positive genotypes with size banding of about 428bp. However, this specific band was absent in the rest of the tested genotypes. Also, NSb dominant marker was linked with *Pib* blast resistant. All tested genotypes were carrying this gene with M.W. of 523 bp, except the genotypes Eg-N-2, Al-Ahsa Type2, and Sakha102. On the other hand, the marker z4794 is a specific co-dominant marker for blast resistant genes *Piz* and *Piz-t*. The results revealed that two alleles were amplified; the first one with molecular weight 204 bp, and the other with molecular weight 141 bp. Nine rice genotypes were positive controls and had the first allele specific bands, while the Egyptian genotypes and the Saudi Arabian were negative control and had the second allele.

pBA14 is a specific dominant marker for rice blast resistance gene *Pi9*. The results showed that none of the genotypes carried this gene, except the genotypes IRBL3-CP4, IRBL5-M and Eg-N-9, with size banding 399 bp. This finding indicated that rice blast resistance gene *Pi9* was absent from all the tested genotypes. In the other SNP marker T8042, which was specific for blast resistant gene *Pit*, the banding pattern of T8042 was presented and the positive control used was Giza177, with a molecular weight of 181 bp. The results showed that all tested genotypes, except Al-Ahsa Type1 and Al-Ahsa Type2 varieties, were negative for the *Pit* gene. According to the last InDel marker K2167, which is a specific marker for rice blast resistant genes *Pik* and *Pik-m*, the monogenic lines IRBLKS were used as positive controls for *Pik* and *Pik-m*, while Sakha101 was used as a negative control. The positive control, as well as five tested genotypes i.e., Eg-N-9, Sakha 102, Sakha 105, Sakha 106 and Giza177, showed the positive amplified band with a molecular weight of 637 bp.

Discussion

The challenges facing the Earth, such as climate change, high temperature, desertification, and the prevalence of diseases, are steadily increasing, and this is having a detrimental effect on the productivity of crops, and therefore on food supplies, thus worsening the global problem of hunger, especially in African countries. For this reason, agricultural research institutions have undertaken breeding programmes aimed to improve agricultural cultivars to overcome these environmental stresses.

Genetic resources with beneficial qualities are an effective tool forr improvement and high productivity of crops such as rice (Garris *et al.,* 2005; Anis *et al.,* 2016). This study focused on the selection of fifteen cultivars of rice from 3 different locations, Egypt, Saudi Arabia and the Philippines, to determine which cultivars are resistant to blast and rich in the nutrients, and therefore qualifies as a distinct genetic source that can be recommended for use by plant breeders. These groups of selected genotypes from geographically and ecologically diverse locations were expected to show wide genetic variations that could be used in the breeding programme as proposed by Olufowot *et al.* (1997). The ordinary analysis of variance for lines, tester and line × tester were shown to be highly significant mean squares for all studied traits, which indicated overall differences among the lines, tester and line × tester (Table 2). These results are similar to those obtained by Faiz *et al.* (2006); El-Wahsh and Hammoud (2007); Rashid *et al.* (2007); Saleem *et al.* (2010); Tiwari *et al.* (2011). Also, mean performance of parents and their F_1 hybrid showed high significance for all studied traits, indicating that rice genotypes differ in their genetic potential (Table 3). High positive values of GCA effects would be of interest in most of the traits under investigation, such as number of tillers/plant, 1000 grain weight (g) and grain yield/plant (Table 4). Generally, the estimates of overall *gca* status of parents indicated the three tester Sakha101, Sakha106, Eg-N-9 out of five lines, while IRBLKS-S as one of the five monogenic lines, were good combiners for most of the traits that were studied, and could be used in breeding programmes for these traits.

The presence of somaclonal variation in populations derived from tissue culture is affecting the use of tissue culture negatively and has remained a major problem (Karp, 1994). Conversely, it can be a useful source for plant breeding via generation of new desirable clones/variants with better disease resistance and agronomic traits under high phytosanitary conditions (Karp, 1995; Unai *et al.,* 2004; Orbovic´ *et al.,* 2008). The nature of gene expression or genetic inheritance for these clones or lines is controlled by genetic dominance, and remains stable in progenies (Roy and Mandal, 2005; Yang *et al*., 2010; Jain *et al*., 2013). The frequency of somaclonal variation is affected by different factors, such as: presence of a disorganized growth phase, nature of explant, growth regulator, number of subcultures, and propagation methods (Pierik, 1987; Rani and Raina, 2000; Bairu *et al.,* 2011). In our experiments, callus for seven genotypes was successfully induced via cultured immature seeds on the MS medium, supplemented with 3 mg L^{-1}2,4-D (Table 5). The healthy calluses were regenerated by added growth regulator BAP at 2.0 mg L^{-1} (Fig. 2). The exact mode of action of 2,4-D is not fully understood, and it is possible that it causes a variety of effects which are fatal when combined. It is believed to acidify the cell walls which allow the cells to elongate in an uncontrolled manner. Low concentrations of 2,4-D can also stimulate RNA, DNA, and protein synthesis leading to uncontrolled cell division and growth, and, ultimately, vascular tissue destruction. Ullah *et al.* (2007) found that the addition of 2,4-D, independently or in combination with BAP, depends mainly on the genotypes. Induction of callus using 2,4-D at high concentration is reported to be associated with genetic abnormalities, such as stimulation of DNA synthesis in different crops (Mohanty *et al*., 2008). Also, Oono (1985) found that high levels of BA have a positive effect on increasing the genetic variability of rice callus cultures. This may refer to the encouragement of the rate of the DNA-methylation process (Loschiavo *et al.,* 1989). In previous studies, somaclonal variants were detected by the use of vegetative, agronomic, yield traits (Sultana *et al.,* 2005; Carsono and Yoshida, 2007) and molecular markers (Salehian *et al*., 2013) in rice. Vegetative and yield traits were used in the recent study to detect the rate of somaclonal variation in the SC1 in the genotypes under study (Table 6). The results relate to the SC1 family derived from Sakha106, Sakha 101, Al-Ahsa Type 1, Al-Ahsa Type 2, Eg-N-1, Eg-N-2 and Eg-N-9 cultivars. The differences between the lines were remarkably slight compared with their parents: these lines could be included in breeding programmes as a genetic resource. Two successive cycles of mature embryo-derived callus cultures, separated by one cycle of sexual reproduction of R_0 regenerated plants were performed using two rice cultivars in order to gain information on the nature of somaclonal variation described by Lutts *et al*. (2001). The latter demonstrated that plants regenerated after one cycle of tissue culture exhibited higher variability and lower performances than those of the initial cultivars. The extent and nature of somaclonal variation depended on the identity of the R_0 mother plant and culture conditions. Larkin *et al.* (1984); De Klerk (1990) advocate the view that somaclonal variation represents a new source of variability and therefore constitutes a powerful tool for the breeder, especially in combination with mutagenic treatment and selection in vitro.

In recent years, many investigations have demonstrated the relationships and the heredity of important mineral element contents, which provide the foundation for plant breeding programmes. The process of genetic selection of high content nutrient "biofortification" using traditional or modern breeding methods is one of the main pillars in the development of micro/macro nutrient staple plant food. Gregorio *et al.* (2000); Graham *et al.* (2001) have evaluated the genetic variability of nutrient concentration in rice grain. There was approximately a 4-fold difference in nutrient concentrations, suggesting some genetic potential and significant genetic diversity in the genome of rice for the increase of the concentrations of these micronutrients in rice grain. Jiang *et al.* (2007) found that significant correlations existed among the contents of some mineral elements in milled rice, and there was a close association between the contents of some mineral elements and three cooking quality traits, 17 amino acid contents or protein content.

Anandan *et al.* (2011) studied the genotypic variation and relationships between quality traits and trace elements in traditional and improved rice genotypes. They found that visible differences could be found in the mineral contents of the rice genotypes studied. The contents of Fe and Zn in traditional genotypes were significantly higher than those of improved cultivars. There was a negative correlation between grain yield and mineral content. The increase in the percentage of the elements in the grains or seeds of the crops was found to lead to higher stimulation of seedling growth when planted in a nutrient- deficient soil. A positive relationship was found between seedling growth strength and root growth and root propagation, which may be resulted in root incursion into larger distances to obtain nutrient and water early in the growth, therefore having a positive effect on yield increase, compared with yield from the cultivation of low-nutrient grains. This explanation is in agreements with (Welch and Graham, 2004). In the recent study, Fe and Zn content appeared to be positively correlated and the genotypes Eg-N -1, Eg-N -2, Eg-N -9 were rich in protein % and micronutrients and could be used to improve the nutritional value of rice, using either conventional or modern biotechnology methods. Our results are in agreement with those of Lang *et al.* (2013), who mentioned that some of the rice cultivars exhibit high grain quality properties, especially of the grain protein content. These could be applied in rice breeding programmes for the purpose of further improvement.

Molecular markers to identify either resistant abiotic genes or resistance biotic gene are employed in many breeding programmes, and offer several advantages (Berruyer *et al.*, 2003; Souframanien and Gopalakrishna, 2006; Jeung *et al.*, 2007; Koide *et al.*, 2009; Miah *et al.*, 2013; El-Malky *et al.*, 2014). SSR and SNP markers have been applied in different genomic experiments for the discovery of new markers, tagging disease-resistant genes and analysing the expression of candidate genes in rice (Collard and Mackill, 2008). Blast resistance genes are crucial for the survival of plants in their fight against rapidly evolving pathogens (Takahashi *et al.*, 2010). In the current study, SSR and SNP approaches were employed to identify genetic markers linked to blast resistance genes, using 15 specific primers. Several resistance genes were determined, and this has helped to increase the accuracy of blast resistance breeding in rice. Different polymorphic bands were observed using SSR, and the results agreed with those of Zietkiewicz *et al.* (1994); Fan *et al.* (2017) which showed that SSR had a higher capacity to reveal polymorphism than other molecular markers. Based on the number of blast resistant genes identified for each genotype, in accordance with SSR analysis, the 15 groups were divided into three: a group carrying three resistance genes (Sp7, Eg-N-1, Eg-N-2, Al-Ahsa Type 1, Al-Ahsa Type 2 and Sakha 102); a group carrying two resistance genes (Eg-N-3, Sakha 101, Sakha 105, Sakha 106, Giza 178, Giza 177, IRBLKS and IRBL5-M); and a group carrying 1 resistance genes (IRBL3-CP4). This results indicated that pyramiding of different resistance genes against blast diseases, using PCR- based different markers linked with different blast resistance genes, may be an effective way of improving the resistance of the rice variety through breeding programmes.

The current study highlighted the importance of producing nutritionally improved rice genotypes. This will help to improve the food security around the world. Plant tissue culture techniques were approved as an effective methodology for the induction of somaclonal variation in rice. This technique was applied for the production of new and nutrient-rice varieties of rice relatively short time compared with the traditional methods of rice breeding. Genes responsible for blast-resistant in rice were identified. This is considered to be an important step toward producing blast-resistant varieties of rice through applying the genetic engineering techniques.

Conclusion

The best genotypes, for grain yield, were IRBLKS-S, Sakha106, Eg-N-9 and Sakha101; whereas for nutrients and protein percentage, the genotypes Eg-N-1, Eg-N-2 and Eg-N-9 had the highest value and would be valuable materials for in breeding programs. Different variation was also noted among the somaclonal population and identification of different resistance genes against blast is an effective way of improving the resistance of rice varieties. The genotypes Eg-N-1, Eg-N-2, Eg-N-9, Al-Ahsa Type1, Al-Ahsa Type2 and Giza177 could be used for gene pyramiding and gene accumulation to produce durable resistance to blast.

Acknowledgments

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant no. G-207-130-38. The authors, therefore, acknowledge with thanks DSR for technical and financial support.

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(Received 16 April 2018; Accepted 22 May 2018)