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EVALUATION OF SILICA NANOPARTICLES (SiO_2NP) AND SOMACLONAL VARIATION EFFECTS ON GENOME TEMPLATE STABILITY IN RICE USING RAPD AND SSR MARKERS

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Rice plant is a typical silica plant which absorbs greater quantity of silicon (Si) other than cereals, comprising as high as 10-20 percent in stem and leaves and it was observed that Si is necessary for normal growth. Furthermore, the silicon was able to decrease transpiration of rice plants (Yoshida *et al*., 1959), to increase the oxidizing power of rice roots. Studies showed that the effects of nanoparticles (NPs) on plants can be beneficial (seedling growth and development) or non-beneficial (to prevent root growth). The propensity of the NPs to cross barriers and their interaction with intracellular structures was found to be owing to their small size and high surface reactivity which contribute to potential cellular and genetic toxicity by the induction of oxidative stress (Adhikari *et al*., 2013).

In comparison to bulk materials, nanoparticles may be more toxic and or beneficial and they have the potential ability of passing the cell membrane of plant because of their general size between 1 to 100 nm. SiO_2 nanoparticles are one of the major and frequently used engineered oxide nanoparticles (Adhikari *et al*., 2013).

Tissue culture generates a wide range of genetic variation in plant species which can be incorporated in plant breeding programs. By *in vitro* selection, mutants with useful agronomic traits, e.g. salt

or drought tolerance or disease resistance, can be isolated in a short duration. The successful use of somaclonal variation is very much dependent on its genetic stability in the subsequent generations for which molecular markers such as RAPDs, AFLPs, SSRs and others can be helpful. The potential of somaclonal variation has yet to be fully exploited by breeders, even though a few cultivars have been developed in crops such as rice (Jain, 2001).

Recently, advances in molecular biology have led to the development of new tools for detecting genetic alteration in response to toxic chemicals tolerance at the level of DNA sequence and structure. DNA based techniques (RFLP, QTL, RAPD, AFLP, SSR and VNTR) are used to evaluate the variation at the DNA sequence level (Cenkci *et al*., 2009). Random amplified polymorphic DNA (RAPD) is used extensively for species classification, genetic mapping and phylogeny etc. in addition, their use in surveying genomic DNA for evidence of various types of DNA damage and mutational events (e.g., rearrangements, point mutation, small insert or deletions of DNA changes) in cells of bacteria, plants and animals (Atienzar *et al*., 2000; Cenkci *et al*., 2010; Cambier *et al*., 2010).

Molecular marker based genetic studies on drought in rice have been carried out with several traits such as root characters, osmotic adjustment, cell membrane stability, relative water content, leaf rolling, stomatal conductance and grain yield (Lanceras *et al*., 2004). Simple sequence repeat is an important tool for genetic variation identification of germplasm and being widely applied in genetic diversity analysis, molecular map construction and gene mapping (Zhang *et al*., 2007; Ma *et al*., 2011)

The present study was carried out to determine the effect of silica nanoparticles as a mutagenic material and somaclonal variations on genome template stability to select drought-tolerant varieties of rice (*Oryza sativa* L.). Also, assessment of DNA changes induced by SiO_2NP and somaclonal variations using two SSR markers associated with drought tolerance trait was the other aim of this study.

MATERIALS AND METHODS

This study was conducted at Genetics Department Laboratory, Faculty of Agriculture, Kafrelsheikh University, Egypt.

Rice material

Seeds of four commercial rice (*Oryza sativa* L.) varieties differ in their response for drought; Sakha-107 (tolerant), Giza-179 (tolerant), Sakha-106 (sensitive) and Sakha-101 (sensitive) from Rice Research and Training Center, Sakha, Egypt were used as sources of mature embryos for germination experiment using SiO_2NP and optimization of the tissue culture system. Seeds were kept in a dry place in the dark under room tempera-

ture before use. Rice seeds were dehulled manually and exposed to running water for 15 min., then immersed in 20 ml water in addition to 5 drops of tween 20 for 15 min. and washing under tab water. Seeds were surface sterilized with 75% ethanol for 5 min., and then were soaked 3 times with sterile distilled water followed by addition of 25% Clorox for 30 min. and rinsed at least seven times with sterile distilled water.

SiO_2 nanoparticles (SiO_2NP) preparation

The SiO_2NP were purchased from NanoTech Egypt Company, City of 6 October, Al Giza, Egypt with a purity of 99.5% and average particle size of < 100 nm. The SiO_2NP were suspended directly in distilled water and dispersed by ultrasonic vibration (100 W, 40 kHz) for 30 min. Small magnetic bars were placed in the suspension for stirring before use to avoid aggregation of the particles.

Germination

After seed sterilization and to ensure surface sterility, seeds were germinated in tubes containing 25 ml of MS (Murashige and Skoog, 1962) free hormone media supplemented with three different concentrations of SiO_2NP in addition to control (0, 150, 300 and 450 ppm) for 21 days. Each treatment was replicated three times with five tubes/replica. The seeds were incubated at 25±2°C in culture room for five days in the dark, then transferred under light condition to germinate and develop roots. After germination, fresh weight, shoot length, root length and number of roots/seedling were measured.

Callus induction

Mature seeds as initial explants from the tested four genotypes were used for starting callus induction. After sterilization, mature embryos were placed on callus induction media and incubated in the dark at 25±2°C for 21 days. Callus induction medium containing MS (Murashige and Skoog, 1962) was incorporated with 100 mg/L my-inositol, 2 mg/L 2,4-D, 3 mg/L NAA, 0.5 mg/L KIN, 40 gm/L sucrose and solidified by 2.5 gm/L phytagel. At the end of 21 days of mature embryo culture, callus induction (%) was calculated for the four rice genotypes.

Plant regeneration

After callus formation at the end of 21 days, regeneration medium was used for initiating root and shoot and maintained for 4 weeks at 25±2°C in 16 h. light. Green spots were observed in the calli after 4-5 days of incubation on regeneration medium containing MS supplemented with 2 mg/L KIN, 2 mg/L BAP, 0.5 mg/L NAA, 40 gm/L sucrose and solidified with 2.5 gm/L phytagel. Regeneration (%) was defined as the number of calli with shoots to the number of cultured calli per Petri dish × 100 after 3-4 weeks. The number of regenerated shoots per callus above or below 1.5 cm was calculated for each callus forming shoots.

Statistical analysis

Each treatment was conducted with three replicates and analyzed by Least Significant Difference (LSD 5%) using SXW software and the results were presented as mean values.

Isolation of genomic DNA, used primers and amplification condition

Genomic DNA was isolated from bulks of three replicates with five seedlings from control and the three treatments of SiO_2NP in addition to one bulk from 15 somaclonal variants from each genotype using Cetyl trimethyl ammonium bromide (CTAB)-based procedure (Murray and Thompson, 1980). DNA amplification was carried out by PCR technique using 5 standard decamer oligonucleotide RAPD primers including:

Primers	Sequance
OPH-01	GGTCGGAGAA
OPH-02	TCGGACGTGA
OPH-03	AGACGTCCAC
OPH-04	GGAAGTCGCC
OPH-05	AGTCGTCCCC

in addition to two specific SSR markers shown in Table (1).

For RAPD primers, amplification reactions were performed as described by Aboulila (2016). For SSR primers, the total volume of reaction mixture (20 µl) consisted of 10 µl 2X PCR Master mix solution [(i-TaqTM) iNtRON Biotechnology, Korea], 1 µl of each primer 10 pmol/µl, 7 µl of double distilled water and 40 ng of template DNA. The reaction was carried out in a thermal cycler (Perkin Elmer Cetus) programmed. The pre-denaturation with 94°C for 5 min, following by 45 cycles of denaturation at 94°C for 1 min, primer annealing at 62°C for 1 min, and primer extension at 72°C for 1.5 min, and then, final extension at 72°C for 7 min. The PCR products were determined on 1.5 and 3% agarose gels for RAPD and SSR markers, respectively by electrophoresis technique and visualized on Benchtop UV-transilluminator and photographed using photoDoc-ItTM Imaging System (Terra Universal, Inc - California, USA). The molecular size of the amplified products was determined against 1 Kb plus DNA ladder (TIANGEN) and O'GeneRuler DNA Ladder Mix, ready-to-use (Thermo Scientific).

Estimation of genomic template stability

Analysis of DNA variations was determined by scoring present (1) or absent (0) of RAPD bands for the bulked samples of each treatment (three treatments of SiO_2NP and one treatment of somaclonal variants) in addition to control. Polymorphism observed in the DNA profiles included the disappearance of normal bands and the appearance of new bands in treated samples when compared with non-treated ones (set to 100%) (Atienzar *et al.*, 1999). The GTS was calculated as follow:

$$\mathrm{GTS}\ (\%) = (1 - \mathrm{a/n}) \times 100$$

Where; a is the number of polymorphic bands detected in each treated sample which is equal to the sum of disappear-

ance of normal bands and appearance of new bands, n is the number of total bands in the control.

RESULTS AND DISCUSSION

Effect of SiO_2NP on germination of rice seeds and growth characters

Since roots are the first target tissue to confront with excess concentrations of pollutants, toxic symptoms seem to appear more in roots rather than in shoots. On the basis of several studies, the following are the principal factors that influenced toxicity in plants: concentration of NPs, particle size and specific surface area, physiochemical properties of NPs, plant species, plant age/life cycle stage, growth media, NP stability, and diluting agents. In this study, seed germination was not affected by application of the three different concentrations of SiO_2NP. All treatments led to 95-100% germination of seeds showing that SiO_2NP did not adversely affect the seed germination and this result in the same line with Adhikari *et al*. (2013). The four studied genotypes have a dose-dependent response for all growth characters as shown in Table (2). It was observed that; with increasing SiO_2NP concentration, the root and shoot growth was also increased in most cases. However, after certain concentrations in few cases, the growth of root and shoot was found to decline. Among the four rice variety seedlings, the best growth response for root length (20.5 cm) was observed at a concentration of 300 ppm over control for Giza-179, while the best growth response for fresh weight (232 mg), shoot length (46.27 cm) and number of roots/seedling (20) were recorded for Sakha-107 in control treatment. At the highest concentration (450 ppm) the growth of rice seedlings was observed over the control for the sensitive-drought genotypes Sakha-101 in fresh weight, shoot length and number of roots/seedling. The reduction in root and shoot growth at higher doses may be attributed to toxic level of nanoparticles (Adhikari *et al*., 2013). This is a good evidence for demonstrating that rice seedlings responded to added nanoparticles in a limited range, above which toxic levels are reached causing subsequent declines in growth.

Callus induction and plant regeneration in rice

Our experimental results revealed highly significant differences among the tested genotypes for all studied parameters as shown in Table (3) and Fig. (1). The highly drought-tolerant rice variety Sakha-107 gave the higher value for callus induction (%), regeneration (%) and the mean number of regenerated shoots/callus. Also, drought-sensitive rice variety Sakha-106 recorded the lowest values for all the studied traits.

The high-quality calli were yellow or light yellow in color and dry, compact, and globular in appearance. On the basis of this morphological criterion of the initiated calli, the combination of 2 mg/l, 2,4-

D, 0.5 mg/l kinetin and 3 mg/l NAA proved to be the most beneficial for callus induction. These calli were dry, compact, light yellowish, and nodular in appearance. Differences were observed among the four rice genotypes for regeneration (%), while the highest differentiation rate was recorded for Sakha-107. Our results have demonstrated that the composition of basal medium used for callus induction also had a significant effect on callus regeneration. Similar results were found by Visarada *et al.* (2002), who showed that the regeneration response was also determined by the induction medium.

Plant growth regulators play a central role in plant tissue culture, in which a high auxin/cytokinin ratio usually is used for initiation of the embryogenic callus, while a low ratio is used for the regeneration of plantlets. Although the exact functional mechanism of plant growth regulators in tissue culture remains unclear, it is suggested that they function by mediating the signal transduction cascade that leads to reprogramming of the expression of embryogenic genes (Dudits *et al.*, 1995). Auxins, especially 2,4-D, are essential for induction and proliferation of the callus, but they also prevent precocious regeneration, which could lead to the loss of embryogenic competence. Cytokinin may increase the growth rate of preoembryogenic masses (Kommamine *et al.*, 1992). In most cases, 2,4-D as a strong synthetic auxin was sufficient to initiate and sustain embryogenic callus growth in rice and has been used as the only growth regulator in callus induction media (Lee *et al.*, 2002; Ozawa *et al.*, 2003; Lin and Zhang, 2005). However, there were also few reports that the combination of 2,4-D with kinetin was more effective in producing an embryogenic callus while 2,4-D alone only produced a non-embryogenic one (Fan *et al.*, 2002; Wang *et al.*, 2004). Other studies showed that the addition of NAA and/or IAA could enhance the quality of the initiated callus (Tian, 1994; Trejo-Tapia *et al.*, 2002). Our results showed that the formation of callus was regulated largely by the type and level of the plant growth regulators present in the culture medium.

Estimation of genome template stability using RAPD markers

To evaluate the genetic effects of SiO_2NP and somaclonal variations in this study, RAPD analysis was performed on DNA extracted from bulks of three replica with 5 seedlings from each treatment (0, 150, 300 and 450 ppm SiO_2NP) in addition to one bulk from 15 somaclonal variants. Five 10-mer oligonucleotide RAPD primers were utilized to discriminate controls from rice plants treated with SiO_2NP and tissue culture-derived plants.

These primers were able to generate reproducible RAPD bands with template DNA from control and treated rice plantlets. The RAPD analysis yielded 3-13 bands in control in the different used genotypes and the total numbers of amplified bands from the five primers were identified in control treatment ranging from 25 to 51 bands for each in Sakha-106 and

Sakha-107, respectively (Table 4 and Fig. 2). Polymorphism was calculated as the presence and/or absence of DNA fragments between the samples. Different polymorphic bands were detected at each concentration of SiO_2NP for the different primers in each rice variety. In all cases, the detected values of GTS% were decreased with increasing SiO_2NP concentration in all genotypes. On the other hand, the value of GTS% for somaclonal variation treatment was lower than the value of GTS% of all SiO_2NP concentrations in all genotypes.

Previous studies have showed that changes in band patterns observed in DNA fingerprint analyses reflected DNA alterations from single base changes (point mutations) to complex chromosomal rearrangements (Atienzar and Jha, 2006; Ozturk *et al*., 2010). The changes that were appeared in the DNA patterns as a result of SiO_2NP and tissue culture treatments were loss of normal bands and appearance of new bands in comparison with the normal control plants. Almost, all used primers produced more than one such alteration in a given sample. The maximum number of disappearing RAPD bands was found at somaclonal variations treatment followed by the higher concentration (450 ppm) of SiO_2NP in the two drought tolerant genotypes (Sakha-107 and Giza-179). But in the drought sensitive genotypes (Sakha-106 and Sakha-101), the maximum number of disappearing RAPD bands was found at the highest concentration (450 ppm) of SiO_2NP followed by somaclonal variations treatment.

The disappearance of bands may be attributed to the presence of DNA photoproducts (e.g. pyrimidine dimers), which can act to block or reduce the amplification of DNA in the PCR reaction (Nelson *et al*., 1996; Atienzar *et al*., 2000). It is suggested that the DNA damage may be serious in the majority of rice seedlings cells exposed to toxic nanoparticle concentrations. At higher SiO_2NP concentrations, it seems that the extent of DNA lesion is so important that the *Taq* DNA polymerase is more often blocked which implies a disappearance of band (depending on the extent of DNA damage). On the other hand, mutations (new annealing events) can only be responsible for the appearance of new bands if they occur at the same locus in a sufficient number of cells. A minimum of 10% of mutations may be required to get new PCR product visible in agarose gel to be amplified by PCR (Atienzar *et al*., 2000). Thus, the new bands could be attributed to mutations while the disappeared bands could be attributed to DNA damage.

Polymorphism as detected by two SSR markers

The SSR marker; RM215 is located on chromosome 9 and associated with panicle length as well as maximum root length (Mccouch *et al*., 2003) (Table 1). This marker amplified number of alleles ranged from 1 to 7 with an average of 3.75 allele/genotype with the major allele size ranging from 154 to173 bp (Table 5 and Fig. 3). This marker showed a polymorphism percentage of 93.75. The expected

product size for allele in this marker was 148 bp (Table 1).

The SSR marker: RM518 is located on chromosome 4 and associated with fresh root weight, root volume and maximum root length (Qu *et al.*, 2008) (Table 1). This marker amplified 1 to 3 alleles/genotypes with an average of 1.75. The size of the major allele is ranging from 162 to 200 bp (Table 5). This marker showed a polymorphism percentage of 75%. The expected product size for allele in this marker is 171 bp (Table 1).

Genetic improvement of rice for drought tolerance through conventional breeding is slow due to the spatial and seasonal variations in drought timing and severity, the complex nature of drought tolerance itself and the difficulty in selecting for combinations of traits which best suit combating drought induced yield reductions (Courtois *et al.*, 2003).

The use of molecular markers to select accessions possessing genes and genomic regions that control target traits can fast-track the progress in breeding for drought tolerant rice, because molecular markers are transmitted faithfully from generation to generation and are not subject to environmental influences (Crouch and Ortiz, 2004).

Results of the present study concluded that (1) the RAPD analysis is a highly sensitive method for the detection of DNA changes induced by SiO_2NP and somaclonal variations, (2) root growth is the most sensitive parameter to SiO_2NP, (3) highly SiO_2NP concentrations and somaclonal variations affect the genomic template stability and may induce gain or loss in the number of bands and (4) the genome template stability is a highly sensitive parameter compared with the traditional indices such as root growth and shoot length. The two SSR markers were successful for identifying variations induced by SiO_2NP and somaclonal variations. This study suggests that the RAPD and SSR analyses used in conjunction with other biomarkers from higher levels of biological organization such as growth parameters can be a powerful tool for identifying DNA changes induced by SiO_2NP and somaclonal variations, which may be useful for induction of drought tolerant rice genotypes.

SUMMARY

Assessment of DNA changes and mutations at molecular level are important in plant breeding. In this study DNA changes in four rice genotypes (Sakha-107, Giza-179, Sakha-106 and Sakha-101) induced by silica nanoparticles (0, 150, 300 and 450 ppm) and somaclonal variations were determined using RAPD and SSR analyses. The potential effects of SiO_2NP (< 100 nm) on rice plant growth were studied and the results showed positive and negative effects. Application of SiO_2NP enhanced the fresh weight, shoot length and root length of the drought-sensitive rice varieties, but number of roots/seedling was increased with high concentration (450 ppm) in all studied genotypes. Mature embryos of four rice

genotypes were used as explant source for callus induction and plant regeneration system. The obtained results showed significant effect of genotype on callus induction and plant regeneration in rice. For assessment of genome template stability percentage (GTS%), five RAPD primers were used and produced a total number of bands ranged from 25 to 51 in the studied genotypes. Results indicated that the GTS% was lower in somaclonal variations for all studied genotypes. Also, the changes occurred in DNA included gain or loss of bands compared with the control plants. On the other hand, among different concentrations of silica nanoparticles, the drought tolerant genotypes (Sakha-107 and Giza-179) gave the highest percentage of GTS in 150 ppm, while the drought sensitive genotypes (Sakha-106 and Sakha-101) gave the highest percentage on 300 and 450 ppm for Sakha-106 and sakha-101, respectively. These results confirmed the effects of SiO_2NP and somaclonal variations on mutation and DNA instability and suggested that genomic template stability (GTS) reflecting changes in RAPD profiles was the most sensitive endpoint compared with the traditional indices such as root and shoot growth. On the other hand, two SSR markers (RM215 and RM518) were applied and generated 93.75 and 75% polymorphism, respectively. These variations among rice varieties and their treatments could help in rice plant breeding for drought tolerance by the selection of the suitable genotypes which are able to tolerate high drought stress conditions.

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Table (1): Details of SSR markers used for molecular characterization.

No.	SSR Loci	Chr. No.	Sequences 5′→3′	Expected PCR product size (bp)	Trait	Reference
1	RM215	9	F:CAAAATGGAGCAGCAAGAGC R:TGAGCACCTCCTTCTCTGTAG	148	MRL	Hittalmani *et al.* (2003)
					PL	McCouch *et al.* (2003)
2	RM518	4	F: CTCTTCACTCACTCACCATGG R:ATCCATCTGGAGCAAGCAAC	171	MRL RFW RV	Qu *et al.* (2008)

MRL: Maximum root length, PL: Panicle length, RFW: Root fresh weight, RV: Root volume.

Table (2): Effect of SiO_2NP (<100 nm) on rice growth characters.

Traits	Genotypes	SiO_2NP concentrations				
		Control	150 ppm	300 ppm	450 ppm	L.S.D.$_{0.05}$
Fresh weight (mg)	Sakha-107	232.0	174.7	138.1	169.2	3.04
	Giza-179	165.7	185.9	188.9	152.5	3.76
	Sakha-106	172.7	165.9	151.7	132.9	6.21
	Sakha-101	202.2	160.8	147.2	208.0	6.89
L.S.D.$_{0.05}$		4.57	5.30	4.94	5.99	----
Shoot length (cm)	Sakha-107	46.27	23.77	20.82	25.13	3.06
	Giza-179	22.83	21.20	20.25	18.38	1.97
	Sakha-106	28.00	13.42	29.44	21.42	1.58
	Sakha-101	20.62	19.95	22.15	21.80	1.12
L.S.D.$_{0.05}$		2.89	1.58	1.93	1.54	----
Root length (cm)	Sakha-107	10.64	14.47	8.62	9.20	0.99
	Giza-179	8.17	16.50	20.50	7.00	1.21
	Sakha-106	10.57	5.25	8.43	7.47	1.17
	Sakha-101	10.03	10.87	11.47	9.48	0.91
L.S.D.$_{0.05}$		1.28	1.02	0.88	1.09	----
Number of roots/seedling	Sakha-107	20.00	9.12	7.00	9.28	1.26
	Giza-179	9.67	9.00	8.33	10.17	1.89
	Sakha-106	7.50	4.67	7.00	8.17	1.13
	Sakha-101	8.50	7.00	8.00	9.25	1.03
L.S.D.$_{0.05}$		1.38	1.24	1.65	1.16	----

Table (3): Effect of culture media on tissue culture parameters in the tested four rice varieties.

Genotypes	Callus induction%	Regeneration%	Mean numbers of regenerated shoots/callus
Sakha-107	96.36 ^{a}	76.43 ^{a}	5.29 ^{a}
Giza-179	92.55 ^{ab}	63.29 ^{b}	3.66 ^{b}
Sakha-106	82.95 ^{b}	31.17 ^{d}	1.41 ^{c}
Sakha-101	86.18 ^{ab}	47.62 ^{c}	1. 72 ^{c}
L.S.D.$_{0.05}$	0.12	5.03	0.51

Table (4): Genome template stability %, the total number of bands in control and polymorphic bands in for three different concentrations of SiO_2NP and somaclonal variations treatment (bulk S.V.) using five RAPD primers in the studied four rice genotypes.

Genotype	Primer name	Total number of control bands	SiO_2NP concentrations (ppm)							
			150		300		450		Bulk S.V.	
			a	B	a	b	a	b	a	b
Sakha-107	OPH-01	13	2	5	1	3	4	5	5	7
	OPH-02	8	0	0	1	0	1	0	1	0
	OPH-03	10	1	0	1	0	1	1	3	0
	OPH-04	11	3	1	2	4	2	4	3	4
	OPH-05	9	1	0	2	1	0	1	2	1
Total bands		51	7	6	7	8	8	11	14	12
a+b			13		15		19		26	
GTS%			74.51		70.59		62.75		49.02	
Giza-179	OPH-01	11	0	0	3	1	3	0	7	4
	OPH-02	5	2	0	4	0	5	0	6	0
	OPH-03	9	0	0	0	0	0	3	1	0
	OPH-04	7	1	1	4	2	4	2	4	2
	OPH-05	8	3	2	2	0	3	3	3	2
Total bands		40	6	3	13	3	15	8	21	8
a+b			9		16		23		29	
GTS%			77.50		60.00		42.50		27.50	
Sakha-106	OPH-01	6	2	0	4	1	2	2	5	2
	OPH-02	4	0	0	0	0	0	0	0	0
	OPH-03	3	2	1	5	0	4	1	7	1
	OPH-04	5	3	0	2	0	3	3	2	0
	OPH-05	7	0	0	3	0	1	3	0	3
Total bands		25	7	1	14	1	10	9	14	6
a+b			8		15		19		20	
GTS %			68.00		40.00		24.00		20.00	
Sakha-101	OPH-01	6	3	0	3	1	0	1	1	1
	OPH-02	5	0	0	0	1	1	0	0	0
	OPH-03	7	3	0	1	1	2	3	3	3
	OPH-04	6	2	0	2	2	1	5	3	4
	OPH-05	11	1	0	4	0	1	4	2	2
Total bands		35	9	0	10	5	5	13	9	10
a+b			9		15		18		19	
GTS%			74.29		57.14		48.57		45.71	
GTS% average			73.58		56.93		44.46		35.56	

a: appearance of new bands, b: disappearance of normal bands, a+b: denotes polymorphic band.

Table (5): Genetic diversity in rice genotypes using two SSR markers.

No.	SSR Loci	Genotypes	Total number of bands	Polymorphism %	Major allele frequency/size
1	RM215	Sakha-107	7	100	0.6 (154 bp)
		Giza-179	4	75	1.0 (173 bp)
		Sakha-106	3	100	0.6 (154 bp)
		Sakha-101	1	100	0.8 (173 bp)
Average			3.75	93.75	0.75
2	RM518	Sakha-107	1	0.00	1.0 (171 bp)
		Giza-179	2	100	0.8 (162 bp)
		Sakha-106	3	100	0.6 (171 bp)
		Sakha-101	1	100	0.8 (200 bp)
Average			1.75	75	0.8

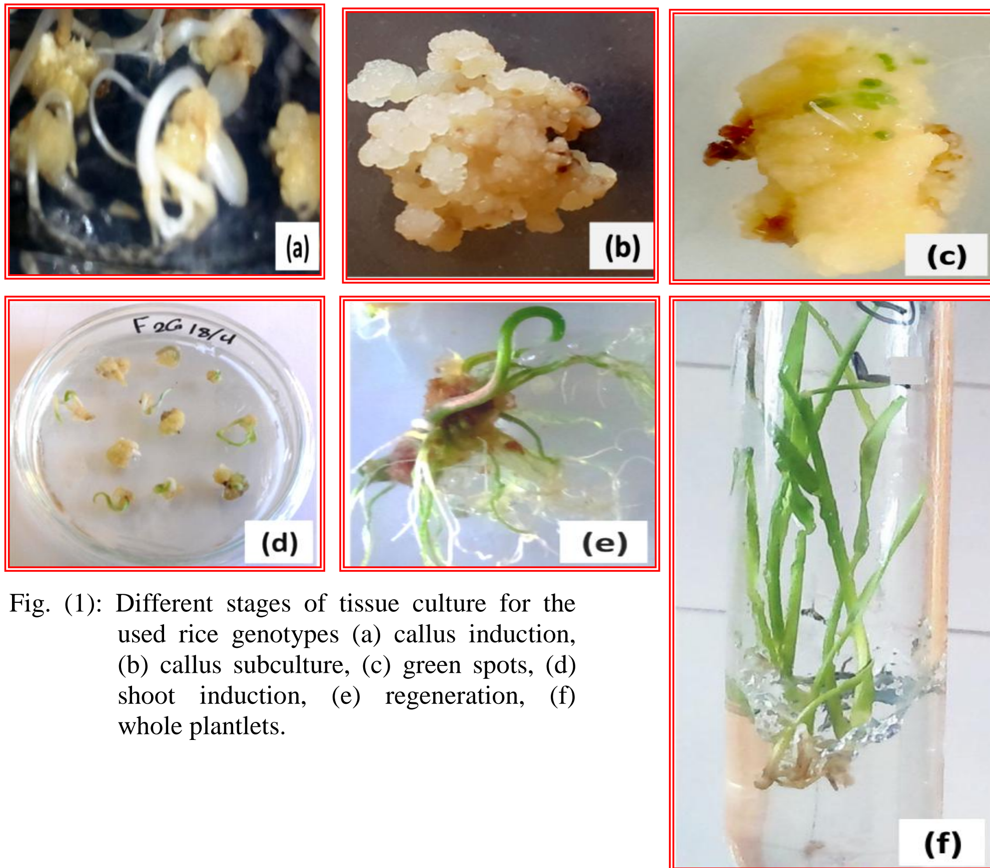

Fig. (1): Different stages of tissue culture for the used rice genotypes (a) callus induction, (b) callus subculture, (c) green spots, (d) shoot induction, (e) regeneration, (f) whole plantlets.

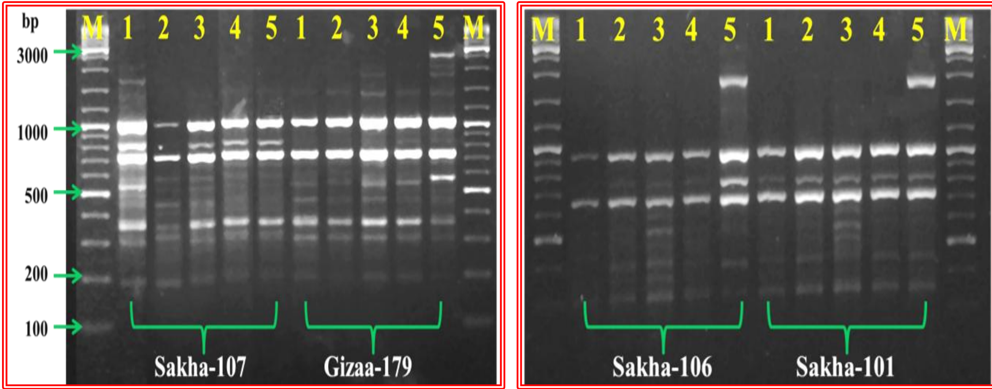

Fig. (2): Amplification pattern of rice genotypes with OPH-01 RAPD marker. (M) O'GeneRuler DNA Ladder Mix, ready-to-use, (1) control, (2), (3), (4) 150, 300, 450 SiO_2NP ppm treatments, respectively and (5) Somaclonal variations treatment.

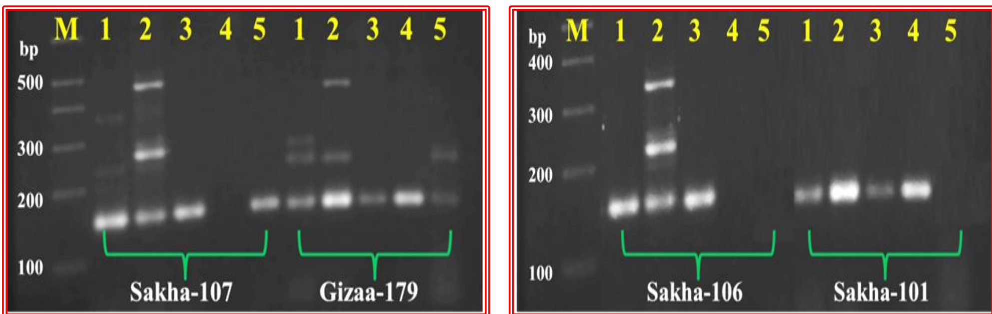

Fig. (3): Amplification pattern of rice genotypes with RM215 marker. Expected PCR product size 148 bp. (M) 1 Kb DNA Ladder, (1) control, (2), (3), (4); 150, 300, 450 SiO_2NP ppm treatments, respectively and (5) somaclonal variations treatment.