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Genetic improvement for drought tolerance in rice using mutation induction

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ABSTRACT

Thirty-three percent of the world's farmland is subject to drought, making it the most difficult abiotic stress on rice production. Ten different M_4-rice mutants were tested, along with three check varieties (Giza 179, Sakha 107, and IET1444 - International check variety for drought stress), to see how well they fared in drought conditions. These genotypes were tested in well-watered (WW: irrigation every 4 days), water-stressed (WS1: irrigation every 8 days), and severe water-stressed (WS2: irrigation every 12 days) conditions across generations M_5 to M_8. Drought stress was measured regarding its effect on agronomic traits and drought tolerance indices. Of the ten tested mutants, seven high-tillering mutants had higher yields under normal and stress conditions than the check varieties did in the field. The STI, MP, YI, and GMP indices show that, compared to IET444 (DT check variety), the mutant EN25 performed best under drought stress, followed by the mutant EN27. According to the data analysis of SCoT markers, only 34 of the 46 primers used amplified 377 bands (alleles) across 53 different markers. There was a wide range of genetic similarities among mutants, parents, and the check varieties, and it ranged from 17% to 78%. These seven mutants shared 13 common bands with the most drought-tolerant check variety (IET444) using SCoT markers, which indicates that these mutants carried some drought-tolerant genes. Hence, these mutants hold great potential for use in drought-stressed rice breeding programs.

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1 Introduction

Rice, *Oryza sativa* L is the staple food source of more than half of the world's population (Rasheed et al. 2020). Rice is the second largest source of calories in the human diet after wheat, accounting for 20% of the total dietary energy supply worldwide (Babaei et al. 2011). Furthermore, it is grown on about 164 million hectares around the world, with a total production of 756.7 million tonnes. Many restrictions exist in Egypt due to limited water resources; rice is grown on approximately 0.66 million hectares each year, yielding 5.5 million tonnes (FAO 2020). Producing promising drought-tolerant rice cultivars with high yielding is one of the main targets for rice breeders. Drought stress is a severely limiting factor to rice production and quality, which decreased the agronomic traits and rice yields by 53-92% (Lafitte et al. 2007). Drought stress affects more than one-third of the world's total cultivated area. Drought resistance is a plant's ability to produce its maximum economic yield when water is scarce (Moussa 2011; Rollins et al. 2013). It is a complex trait that is determined by the action and interaction of various morphological, biochemical, and physiological responses. Breeding for drought-tolerant rice varieties is a thought-provoking task because of its complex nature and multigenic control.

Breeding rice varieties that are resistant to drought stress provides an economically viable and long-term solution for increasing rice productivity (Pandey and Shukla 2015). Mutation breeding has proven to be an effective method for introducing new traits that may lead to crop improvement and can be used in conjunction with plant breeding (Babaei et al. 2010). Mutation induction techniques can be utilized for crop improvement through increasing genetic diversity, which enables plant breeders to select according to the desired breeding objectives (Abdul Haris et al. 2013). Mutation breeding involves developing new varieties characterized by abiotic stress tolerance, early maturity, and high productivity using physical and chemical mutagens (Oladosua et al. 2016). Gamma rays have been successful in inducing genetic variability in rice. The mutant variety database contains more than 3,364 mutants, mainly consisting of cereal species (47.13%) with 851 mutants in rice crops, among them 248 tolerant to abiotic stress (FAO 2020). Molecular markers are effective tools for assessing genetic variation and elucidating genetic relationships within and between species (Chakravarthi and Naravaneni 2006), increasing the efficacy of selection in breeding programs. Start codon targeted polymorphism (SCoT) is a novel marker system for gene differential expression developed based on the short conserved regions flanking the ATG start codon in the plant genome. These markers can be used to find new genes (Collard and Mackill 2009). So, this study was conducted to evaluate rice mutants for tolerance to drought and salinity stress as well as yield and yield-related traits using gamma rays and molecular techniques.

2 Material and Methods

Ten rice mutants in the M_4 generation, namely EN7, EN14, EN17, EN24, EN25, EN26, EN27, EN28, En32, and EN46, were selected from populations of 2 local cultivars, Giza 178 (Gz178) and Sakha 101 (Sk101) that irradiated with varying doses of gamma rays (0, 200, 250 and 300 Gy). These mutants, the parent of most mutants (Giza 178) and three drought tolerance check varieties (Giza 179; Sakha 107 and IET1444) were used to investigate genetic diversity for drought tolerance. The visual selection was based on drought tolerance, early maturity, and high grain yield.

2.1 Evaluation of tolerance to drought stress

Ten rice mutants, a Gz178 cultivar (parent), and three tolerant check cultivars were evaluated for drought tolerance in the El-Sharkyia Governorate location for four years from M_5 to M_8 generations (2017-2020) under three irrigation intervals: as well-watered (WW) (irrigation every 4-days), water-stressed 1 (WS1) (irrigation every 8-days) and severe water-stressed 2 (WS2) (irrigation every 12-days) conditions in loam soil. The grains of rice genotypes were planted as individual plants in separate rows in a split-plot design with three replications. Plant height (PH), Number of grains per panicle (NGP), Number of panicles per square meter (NPM), and Grain yield per square meter by gram (GYM) were recorded.

2.2 Drought tolerance indices

Drought tolerance indices were calculated by using the following formulas (Afify et al. 2022).

Stress susceptibility index (SSI) = [1- (Ys / Yp)] / [1-($\overline{Ys}$/($\overline{Yp}$)] (Fischer and Maurer 1978). Tolerance index (TOL) = Yp – Ys (Rosielle and Hamblin 1981).

Mean productivity (MP) = (Yp + Ys) / 2 (Rosielle and Hamblin 1981).

Geometric mean productivity (GMP) = $\sqrt{Ys \times Yp}$ (Fernandez 1992).

Stress tolerance index (STI) = (Ys×Yp) / $\overline{Yp}^2$ (Fernandez 1992).

Yield index (YI) = Ys/ $\overline{Ys}$ (Gavuzzi et al. 1997).

Yield stability index YSI= Ys / Yp (Bouslama and Schapaugh, 1984).

Sensitivity drought index SDI= (Yp-Ys) /Yp (Farshadfar and Javadinia 2011).

Relative drought index RDI= (Ys/Yp) / ($\overline{Ys}$/($\overline{Yp}$) (Fischer and Maurer 1978).

Where Ys and Yp represent yield in stress and non-stress conditions respectively. Also, Ys^- and Yp^- are the mean yield of all genotypes in stress and non-stress conditions respectively. Si is the stress intensity and is calculated as $Si= 1- (YS^- / Yp^-)$.

2.3 SCoT analysis

According to Dellaporta et al. (1983), DNA was extracted using a modified CTAB method. Forty-six SCoT markers were used for PCR amplification for the selected rice genotypes (Table 1). PCR reactions were conducted at a final volume of 12.5 µl, containing 6.25 µl PCR master mix (KAPA2G Fast Ready Mix PCR Kit), 1 µl genomic DNA, 1 µl for each primer and 3.25 µl dH2O. The PCR reactions were performed in a thermal cycler (TECHNE TC-412) programmed as follows: 94°C/3 min for pre-denaturation followed by 35 cycles 94°C/1 min, annealing temperature 50°C/1 min, 72°C/2 min), and 72°C/5 min for final extension then held at 4°C. The PCR products were separated by electrophoresis in 1.2% agarose gel at 80 V for 50 min in 1x TAE buffer, stained with ethidium bromide, and visualized on a UV transilluminator.

2.4 Statistical analysis

Analysis of variance for agronomic and yield traits of M_7 and M_8 generations were subjected to the combined analysis of split-plot design with three replications over two years using the statistical software MSTAT-C. Furthermore, the mean comparisons among the treatments were carried out by Duncan's Multiple Range Test (DMRT).

3 Results and Discussion

3.1 Analysis of variance

Analysis of variance for yield and yield-related traits of 14 rice genotypes in the combined analysis under normal and water stress conditions over two years (M_7 and M_8) is presented in Table 2. The results revealed that mean squares due to the watering stress (WS), genotypes (G), and G x WS interaction were significant for all studied traits in two years except the NGP, suggesting that water stress had a significant effect among the mutants and check varieties. The significance of interaction variance indicates that the

Table 1 Description of SCoT markers used in the study

primer	Sequence (5′-3′)	primer	Sequence (5′-3′)
SCoT1	CAACAATGGCTACCACCA	SCoT24	CACCATGGCTACCACCAT
SCoT2	CAACAATGGCTACCACCC	SCoT25	ACCATGGCTACCACCGGG
SCoT3	CAACAATGGCTACCACCG	SCoT26	ACCATGGCTACCACCGTC
SCoT4	CAACAATGGCTACCACCT	SCoT27	ACCATGGCTACCACCGTG
SCoT5	CAACAATGGCTACCACGA	SCoT28	CCATGGCTACCACCGCCA
SCoT6	CAACAATGGCTACCACGC	SCoT29	CCATGGCTACCACCGGCC
SCoT7	CAACAATGGCTACCACGG	SCoT30	CCATGGCTACCACCGGCG
SCoT8	CAACAATGGCTACCACGT	SCoT31	CCATGGCTACCACCGCCT
SCoT9	CAACAATGGCTACCAGCA	SCoT32	CCATGGCTACCACCGCAC
SCoT10	CAACAATGGCTACCAGCC	SCoT33	CCATGGCTACCACCGCAG
SCoT11	AAGCAATGGCTACCACCA	SCoT34	ACCATGGCTACCACCGCA
SCoT12	ACGACATGGCGACCAACG	SCoT35	CATGGCTACCACCGGCCC
SCoT13	ACGACATGGCGACCATCG	SCoT36	GCAACAATGGCTACCACC
SCoT14	ACGACATGGCGACCACGC	SCoT37	CAACAATGGCTACCAGCG
SCoT15	ACGACATGGCGACCGCGA	SCoT38	AAGCAATGGCTACCACCG
SCoT16	ACCATGGCTACCACCGAC	SCoT39	ACGACATGGCGACCAGCG
SCoT17	ACCATGGCTACCACCGAG	SCoT40	ACGACATGGCGACCACGT
SCoT18	ACCATGGCTACCACCGCC	SCoT41	ACGACATGGCGACCGCGG
SCoT19	ACCATGGCTACCACCGGC	SCoT42	ACCATGGCTACCACCGAT
SCoT20	ACCATGGCTACCACCGCG	SCoT43	ACCATGGCTACCACCGGT
SCoT21	ACGACATGGCGACCCACA	SCoT44	GCAACAATGGCTACCACG
SCoT22	AACCATGGCTACCACCAC	SCoT45	CATGGCTACCACCGGCCG
SCoT23	CACCATGGCTACCACCAG	SCoT46	CCATGGCTACCACCGGCA

Table 2 Mean squares for the studied traits of 14 rice genotypes in combined analysis under normal and water stress conditions over two years

S.V	d.f	PH	NPM	NGP	GYM
Years (Y)	1	2.28	16.20	3.02	0.00
Replications (Y)	4	0.19	967.30**	283.5**	0.003
Water Stress (WS)	2	2352.7**	41741.0**	1398.4**	0.883**
Y x WS	2	2.65	20.95	3.33	0.000
Error	8	2.82	128.31	8.14	0.001
Genotypes (G)	13	145.7 **	72050.1**	3301.1**	0.775**
Y x G	13	1.17	0.83	0.44	0.000
GxWS	26	9.009 **	552.29**	24.84	0.012*
Y x GxWS	26	0.48	0.41	0.34	0.000
Error	156	3.10	311.41	117.9	0.007

*, **indicate significant at p≤0.05 and p≤0.01, respectively; PH: Plant height (cm); NTM: Number of panicles per M^2; NFGP: Number of grains per panicle; GYP: Grain yield per M^2(g)

Table 3 Performance of plant height trait among 14 rice genotypes under drought stress over two years

Genotypes	WW	WS1	Change (%)	WS2	Change (%)	Mean
EN7	101.7^{ab}	91.73^{j-m}	9.80	86.73^{pq}	14.72	93.40^{d}
EN14	100.0^{bc}	95.33^{e-h}	4.67	90.00^{l-o}	10.00	95.11^{bc}
EN17	100.9^{a-c}	95.60^{e-g}	5.25	91.27^{k-n}	9.54	95.93^{b}
EN24	100.0^{bc}	95.70^{e-g}	4.30	91.03^{k-n}	8.97	95.59^{b}
EN25	99.43^{b-d}	96.60^{ef}	2.85	91.27^{k-n}	8.21	95.77^{b}
EN26	101.2^{a-c}	93.70^{g-j}	7.41	88.03^{op}	13.01	94.31^{cd}
EN27	99.27^{cd}	94.60^{f-i}	4.70	88.27^{op}	11.08	94.04^{cd}
EN28	103.0^{a}	97.50^{de}	5.34	91.17^{kn}	11.49	97.22^{a}
EN32	100.9^{a-c}	94.73^{f-i}	6.11	89.73^{m-o}	11.07	95.12^{bc}
EN46	92.50^{i-k}	88.50^{op}	4.32	83.17^{s}	10.09	88.06^{g}
Gz178	96.83^{ef}	92.17^{j-l}	4.81	85.17^{q-s}	12.04	91.39^{e}
Gz179	96.57^{ef}	91.07^{k-n}	5.70	84.07^{rs}	12.94	90.57^{ef}
IET1444	93.97^{g-j}	90.97^{k-n}	3.19	85.63^{qr}	8.88	90.19^{ef}
Sk107	93.13^{h-k}	89.30^{no}	4.11	85.80^{qr}	7.87	89.41^{f}
Mean	98.54^{a}±0.57	93.39^{b}±0.458	5.18	87.95^{c}±0.47	10.71	

WW: Well-watered (every 4-days); WS1: Water- stressed (every 8-days);WS2: Water stressed (every12 days); (mean for each treatment ±SE); LSD = 2.011.

rank of mutant differs from well-watering to water stress environment for all studied traits.

3.2 Evaluation of tolerance to drought stress

3.2.1 Plant height (cm)

The study's findings revealed that EN28 and EN17 mutants had the highest average plant height values (97.22 and 95.93 cm, respectively) under drought conditions (Table 3). On the other hand, EN46 and SK107 have the lowest values for PH over two years (88.06 and 89.41 cm, respectively). Drought stress caused a significant reduction in PH traits, ranging from 2.85% to 14.72%, as observed in EN25 and EN7, respectively. The lowest PH reduction (2.85% and 3.19%) was seen for EN25 and IET 1444, respectively, in the WS1 condition. Furthermore, in the WS2 condition, SK107 and EN25 were reduced by 7.87% and 8.21%, respectively. However, EN7 showed a significant reduction (14.72%).

Drought stress decreases metabolic activity because of a shortage of water, which leads to a decrease in turgor pressure, which impacts plant cell division and elongation processes and lowers PH. Furthermore, the decrease in PH can be attributed to a decrease in

gibberellins, which are required for stem elongation and are associated with water stress (Yang et al. 2001). Similar findings were recorded by Yeo (1999), who discovered that a lack of water reduced the PH of rice. Furthermore, Terra et al. (2013) found a significant reduction in PH in upland rice genotypes that were submitted to water deficiency. Also reduction in the PH of rice genotypes under water stress has been reported in numerous studies by Davatgar et al. (2009); Hussain et al. (2018) and Hussain et al. (2021).

3.2.2 Grains yield per square meter

The results presented in Table 4 revealed that drought stress caused a significant reduction in grain yield per square meter in two generations; this reduction ranged from 7.33 to 15.94%. Also, the grain yield/m^2 ranged from 0.933 kg to 1.689 kg per square meter in Gz178 and EN25, respectively, under drought conditions. Although tolerant drought check variety (Gz179) gave the lowest reduction in grain yield (0% and 9.09%) under WS1 and WS2 conditions, with grain yield (1.07 kg per square meter) under WS2 conditions, respectively. The drought-tolerant rice mutants (EN25 and EN27) exhibited the highest mean for grain yield (1.69 and 1.39 kg/m2) under irrigation conditions (WS2 every 12 days). On the other hand, the EN28 mutant was more influenced under WS1 and WS2 conditions since it had the highest reduction percentage in grain yield (26.45%).

The primary important trait for improving drought tolerance is grain yield under stress. Drought stress inhibits rice growth by influencing various traits such as seedling biomass, stomatal conductance, starch metabolism, plant water relations, and photosynthesis. The photosynthesis process is essential to maintain crop growth and development. Furthermore, chlorophyll content is one of the major chloroplast components for photosynthesis, and it has a positive relationship with photosynthetic rate. As a result, decreased chlorophyll content due to water stress may produce reactive oxygen species (ROS), which can lead to chlorophyll destruction (Ahmadikhah and Marufinia 2016; Sarkarung et al. 1997; Quampah et al. 2011). Furthermore, Pantuwan et al. (2000) revealed that depending on the timing, length, and intensity of the plant water stress, it was found that under drought conditions, the grain production of some rice cultivars could drop by up to 81%.

3.2.3 Number of panicles per square meter

The results presented in Table 5 showed that drought stress caused a significant reduction in the number of panicles per square meter in two generations, ranging from 5.69% to 10.97%. Also, the number of panicles per square meter ranged from 309.2 in EN28 to 531.5 in EN25. Minimum reduction in (NPM) was shown in EN28 (1.47%) by WS1 and 3.03% by WS2, while maximum reduction (17.60%) was reported in EN46.

Ahmadikhah and Marufinia (2016) found that severe water deficit compared to normal irrigation significantly reduced the tiller number in rice. Under water stress conditions, effective tiller production may be reduced due to a limited supply of assimilates, less water uptake to prepare sufficient food, and inhibition of meristematic tissue cell division (Zubaer et al. 2007). Furthermore, the decrease in the number of tillers could be due to decreased photosynthesis and plant growth (Quampah et al. 2011).

Table 4 Performance of grain yield trait among 14 rice genotypes under drought stress over two years

Genotypes	WW	WS1	Change (%)	WS2	Change (%)	Mean
EN7	1.433^{c-e}	1.233^{f}	13.96	1.200^{fg}	16.26	1.289^{d}
EN14	1.133^{f-i}	1.067^{h-k}	5.83	1.000^{j-l}	11.74	1.067^{fg}
EN17	1.233^{f}	1.167^{f-h}	5.35	1.067^{h-k}	13.46	1.156^{e}
EN24	1.400^{de}	1.333^{e}	4.79	1.233^{f}	11.93	1.322^{cd}
EN25	1.800^{a}	1.733^{a}	3.72	1.533^{bc}	14.83	1.689^{a}
EN26	1.167^{f-h}	1.067^{h-k}	8.57	0.9667^{kl}	17.16	1.067^{fg}
EN27	1.567^{b}	1.400^{de}	10.66	1.200^{fg}	23.42	1.389^{b}
EN28	1.133^{f-i}	0.9333^{lm}	17.63	0.8333^{m}	26.45	0.967^{hi}
EN32	1.167^{f-h}	1.133^{f-i}	2.91	1.033^{i-l}	11.48	1.111^{ef}
EN46	1.100^{g-j}	1.000^{j-l}	9.09	0.9333^{lm}	15.15	1.011^{gh}
Gz178	1.033^{i-l}	0.933^{lm}	9.65	0.8333^{m}	19.33	0.933^{i}
Gz179	1.100^{g-j}	1.100^{g-j}	0.00	1.000^{j-l}	9.09	1.067^{fg}
IET1444	1.467^{b-d}	1.400^{de}	4.57	1.200^{fg}	18.20	1.356^{bc}
Sk107	1.133^{f-i}	1.067^{h-k}	5.83	0.9667^{kl}	14.68	1.056^{fg}
Mean	1.267^{a}±0.036	1.183^{b}±0.034	7.33	1.071^{c}±0.029	15.94	

WW: Well -watered (every 4-days); WS1: Water- stressed (every 8-days); WS2: Water stressed (every12-days);(mean for each treatment ± SE); LSD = 0.09542

Table 5 Performance of the number of panicles per square meter among 14 rice genotypes under drought stress over two years

Genotypes	WW	WS1	Change (%)	WS2	Change (%)	Mean
EN7	448.7^{de}	414.7^{g}	7.58	393.6^{h}	12.28	419.0^{d}
EN14	351.4^{k-m}	324.9^{n-r}	7.54	325.2 $^{n-r}$	7.46	333.8^{h}
EN17	377.2^{h-j}	352.2^{k-m}	6.63	332.5^{m-q}	11.85	353.9^{ef}
EN24	465.4^{d}	441.0^{ef}	5.24	424.5^{fg}	8.79	443.6^{c}
EN25	567.3^{a}	536.4^{b}	5.45	490.8^{c}	13.48	531.5^{a}
EN26	362.3^{i-l}	341.2^{l-n}	5.82	325.3^{n-r}	10.21	342.9^{f-h}
EN27	382.3^{hi}	350.8^{k-m}	8.24	321.5^{n-r}	15.90	351.6^{ef}
EN28	313.9^{p-r}	309.3^{qr}	1.47	304.4^{r}	3.03	309.2^{i}
EN32	354.8^{j-m}	337.5^{m-p}	4.88	314.2^{p-r}	11.44	335.5^{h}
EN46	382.9^{hi}	335.0^{m-p}	12.51	315.5 $^{o-r}$	17.60	344.4^{f-h}
Gz178	353.3^{k-m}	339.7^{l-n}	3.85	319.5^{n-r}	9.57	337.5^{gh}
Gz179	374.0^{h-k}	363.0^{i-l}	2.94	339.1^{l-o}	9.33	358.7^{e}
IET1444	489.60^{c}	469.10^{d}	4.19	435.8^{e-g}	10.99	464.8^{b}
Sk107	367.5^{i-k}	355.2^{j-m}	3.35	324.9^{n-r}	11.59	349.2^{e-g}
Mean	399.3^{a}±10.7	376.4^{b}±10.0	5.69	354.8^{c}±8.7	10.97	

WW: Well-watered (every 4-days); WS1: Water- stressed (every 8-days); WS2: Water stressed (every12-days); (mean for each treatment ± SE); LSD = 20.13

Table 6 Performance of the number of grains per panicle among 14 rice genotypes under drought stress over two years

Genotypes	WW	WS1	Change (%)	WS2	Change (%)	Mean
EN7	142.5^{e-j}	133.0^{h-l}	6.67	130.3^{i-l}	8.56	135.2^{fg}
EN14	166.9^{bc}	156.1^{c-e}	6.47	150.6^{e-g}	9.77	157.8^{b}
EN17	156.5^{c-e}	147.5^{e-h}	5.75	143.8^{e-i}	8.12	149.3^{cd}
EN24	147.0^{e-h}	141.9^{e-k}	3.47	140.3^{f-l}	4.56	143.1^{de}
EN25	154.6^{c-f}	151.4^{d-g}	2.07	150.2^{e-g}	2.85	152.1^{bc}
EN26	154.3^{c-f}	151.3^{d-g}	1.94	150.3^{e-g}	2.59	152.0^{bc}
EN27	185.5 ^{a}	180.6^{a}	2.64	175.6 ^{ab}	5.34	180.6 ^{a}
EN28	144.8^{e-i}	140.4^{f-k}	3.04	140.1^{f-l}	3.25	141.8^{d-f}
EN32	165.8^{b-d}	157.1^{c-e}	5.25	156.9^{c-e}	5.37	159.9^{b}
EN46	133.4^{h-l}	131.2^{i-l}	1.65	127.0^{kl}	4.80	130.6^{g}
Gz178	156.6^{c-e}	150.3^{e-g}	4.02	150.4^{e-g}	3.96	152.4^{bc}
Gz179	143.1^{e-i}	137.1^{g-l}	4.19	137.1^{g-l}	4.19	139.1^{ef}
IET1444	156.6^{c-e}	150.3^{e-g}	4.02	150.4^{e-g}	3.96	152.4^{bc}
Sk107	130.6^{i-l}	127.8^{j-l}	2.14	125.4^{l}	3.98	127.9^{g}
Mean	152.7^{a}±2.5	146.9^{b}±2.4	3.81	144.9^{c}±2.3	5.09	

WW: Well-watered (every 4-days); WS1: Water- stressed (every 8-days); WS2: Water stressed (every12 days); mean for each treatment ±SE; LSD = 12.39

3.2.4 Number of grains per panicle

The results showed that drought stress caused a significant reduction in the number of grains per panicle in two studied generations, ranging from 3.81% to 5.09%, as presented in Table 6. Also, NGP ranged from 127.9 in SK107 to 180.6 in EN27. The lowest reduction of NGP was seen for EN46 (1.65%) in WS1 and were 2.59% and 2.85% in (EN26 and EN25) mutants, respectively in WS2 treatment. However, the highest reduction was reported at 9.77% in EN14.

Under water stress conditions, grain size, number, and ultimately weight were reduced due to decreased water content in the plant, which limits reproductive development and grain growth (Pantuwan et al. 2000). Additionally, decreased NGP under water stress levels as a result of inhibition of assimilating to grains translocation (Zubaer et al. 2007). Cha-um et al. (2010) reported a similar result in rice, reporting differential responses of two tolerant rice genotypes to moisture deficit for fertile grains. These tolerant genotypes were not significantly reduced in NGP, resulting in higher productivity than the two sensitive varieties.

3.3 Drought tolerance indices

Drought tolerance indices were developed to select drought-tolerant genotypes based on grain yield potential in well-irrigated and stress conditions (Bennani et al. 2017). From the obtained

Table 7 Drought tolerance indices of 14 rice genotypes over two years

Genotypes	Yp	Ys	STI	MP	GMP	TOL	SSI	YI	YSI	SDI	RDI
Water Stress 1											
EN7	5.73	4.93	1.09	5.33	5.31	0.80	1.87	1.04	0.86	0.14	0.93
EN14	4.53	4.27	0.74	4.40	4.40	0.26	0.77	0.90	0.94	0.06	1.02
EN17	4.93	4.67	0.89	4.80	4.80	0.26	0.71	0.99	0.95	0.05	1.02
EN24	5.60	5.33	1.15	5.47	5.46	0.27	0.65	1.13	0.95	0.05	1.03
EN25	7.20	6.93	1.92	7.07	7.06	0.27	0.50	1.47	0.96	0.04	1.04
EN26	4.67	4.27	0.77	4.47	4.47	0.40	1.15	0.90	0.91	0.09	0.99
EN27	6.27	5.60	1.35	5.94	5.93	0.67	1.43	1.19	0.89	0.11	0.97
EN28	4.53	3.73	0.65	4.13	4.11	0.80	2.37	0.79	0.82	0.18	0.89
EN32	4.67	4.53	0.81	4.60	4.60	0.14	0.40	0.96	0.97	0.03	1.05
EN46	4.40	4.00	0.68	4.20	4.20	0.40	1.22	0.85	0.91	0.09	0.98
Gz178	4.13	3.73	0.59	3.93	3.92	0.40	1.30	0.79	0.90	0.10	0.98
Gz179	4.40	4.17	0.71	4.29	4.28	0.23	0.70	0.88	0.95	0.05	1.02
IET1444	5.87	5.60	1.26	5.74	5.73	0.27	0.62	1.19	0.95	0.05	1.03
SK107	4.53	4.27	0.74	4.40	4.40	0.26	0.77	0.90	0.94	0.06	1.02
Mean	5.10	4.72	0.95	4.91	4.91	0.39	1.03	1.00	0.92	0.08	1.00
Water Stress 2											
EN7	5.73	4.80	1.06	5.27	5.24	0.93	1.02	1.12	0.84	0.16	1.00
EN14	4.53	4.00	0.70	4.27	4.26	0.53	0.74	0.93	0.88	0.12	1.05
EN17	4.93	4.27	0.81	4.60	4.59	0.66	0.84	1.00	0.87	0.13	1.03
EN24	5.60	4.93	1.06	5.27	5.25	0.67	0.75	1.15	0.88	0.12	1.05
EN25	7.20	6.13	1.70	6.67	6.64	1.07	0.94	1.43	0.85	0.15	1.01
EN26	4.67	3.87	0.69	4.27	4.25	0.80	1.08	0.90	0.83	0.17	0.99
EN27	6.27	4.80	1.16	5.54	5.49	1.47	1.48	1.12	0.77	0.23	0.91
EN28	4.53	3.33	0.58	3.93	3.88	1.20	1.67	0.78	0.74	0.26	0.87
EN32	4.67	4.13	0.74	4.40	4.39	0.54	0.73	0.96	0.88	0.12	1.05
EN46	4.40	3.73	0.63	4.07	4.05	0.67	0.96	0.87	0.85	0.15	1.01
Gz178	4.13	3.33	0.53	3.73	3.71	0.80	1.22	0.78	0.81	0.19	0.96
Gz179	4.40	4.00	0.68	4.20	4.20	0.40	0.57	0.93	0.91	0.09	1.08
IET1444	5.87	4.80	1.08	5.34	5.31	1.07	1.15	1.12	0.82	0.18	0.97
SK107	4.53	3.87	0.67	4.20	4.19	0.66	0.92	0.90	0.85	0.15	1.02
Mean	5.10	4.29	0.86	4.69	4.68	0.82	1.00	1.00	0.84	0.16	1.00

Ys and Yp represent yield (ton/acre) in stress and non-stress conditions, respectively. Also, WS1: Water- stressed (every 8-days); WS2: Severe Water stressed (every 12-days); SSI: Stress susceptibility index; TOL: Tolerance index; MP: Mean productivity; GMP: Geometric mean productivity; STI: Stress tolerance index; YI: Yield index; YSI: Yield stability index; SDI: Sensitivity drought index and RDI: Relative drought index.

mutants in this study, EN24, EN25, EN26, EN27, EN32, and IET1444 (cultivar) had the largest STI, YP, and YS indicating they might be the best promising tolerant. On the other hand, Gz178, EN28, and EN46 were the most susceptible genotypes because they showed the smallest STI by WS1 and WS2 treatments.

These results are in agreement with Moghaddam and HadiZadeh (2002). They found that STI was a more helpful index for identifying genotypes that produce high yields under favorable and water-stress conditions. Further, they recommended that a high value of STI implies higher tolerance to abiotic stress. Similar findings are also documented by Farshadfar et al. (2013); Abdelghany et al. (2016); Eid and Sabry (2019) and El-Hosary et al. (2019).

Rosielle and Hambin (1981) also reported that MP refers to the average yield of genotypes between water stress and well-irrigated. In this study, the genotypes with high values of MP were EN25 (7.07), EN27 (5.94), IET1444 (5.74), and EN24 (5.47); these genotypes were considered tolerant to drought. On the other side, Gz178 cultivar (3.93), EN28 (4.13), and EN46 (4.20) mutants had lower values as presented in table 7. Also, Genotypes EN25, EN27, IET1444, and EN24 exhibited the highest values for GMP indices, therefor these genotypes are drought tolerant, however, cultivar Gz178 (3.93), mutants EN28 (4.11) and EN46 (4.20) were the most susceptible genotypes. Besides EN25, EN27, IET1444, and EN24 were drought-tolerant genotypes based on STI, MP, and GMP indices. While the cultivars Gz178, EN28, and EN46 were found the most sensitive genotypes. Therefore, STI, MP, and GMP are considered more efficient indices in the high selection yielding drought-tolerant genotypes under well-irrigated and water-stress conditions. The same outcomes were reported by Mursalova et al. (2015); Ali and El-Sadek (2016), and Eid and Sabry (2019).

The highest Tol values were related to mutants EN7 and EN28, which recorded values of 0.80 in WS1, while EN27 had the highest Tol value (1.47 in WS2). The high amount of Tol is a sign of susceptibility to stress (Parchin et al. 2013; Eid and Sabry 2019). On the other side, EN32 (0.14), EN24 (0.27), Gz179 (0.23), IET1444 (0.27), EN25 (0.27), EN14 (0.26), EN17 (0.26) and SK107 (0.26) has the lowest values recorded in WS1, and these mutants were considered as tolerant genotypes which, showed a lower value of TOL (stress tolerance). Similar findings were documented by various previous researchers (Raman et al. 2012; Pantuwan et al. 2002; Ouk et al. 2006; Sio-Se Mardeh et al. 2006).

The mutants which showed stress susceptibility index (SSI) values <1 could be considered drought tolerant as compared with those of stress susceptibility index > 1. As shown in Table 7, SSI ranged from 0.40 for EN32 to 2.37 for EN28. The lowest values of 0.40, 0.50, 0.57, 62, and 0.65 were reported for the genotypes EN32, EN25, Gz179 IET1444, and EN24, respectively. So, these genotypes were considered to be more tolerant to drought. These current mutants had the same trend as SDI. These results are in agreement with Kumar et al. (2012). Whereas EN28, and EN7, with high SSI values of 2.37 and 1.87, respectively, can be considered susceptible to drought and only suitable for normal irrigation conditions. Similar results were recorded by Abdi et al. (2013); Raman et al. (2012); Eid and Sabry (2019) and Afiah et al. (2019).

A genotype is deemed suited for drought circumstances if it has a high Yield index (YI) value. The genotype which has>1 value is considered tolerant, while the genotype having <1 value is denoted as a susceptible one. EN25, EN27, IET1444, and EN24 exhibited the highest YI values of 1.47, 1.19, 1.19, and 1.13, respectively, indicating tolerant genotypes as in the case of STI cross-testing of genotypes suitable for drought conditions. Similarly, lower values of YI were noted in the genotypes that exhibited susceptibility to drought, while all other genotypes were intermediate. The highest YSI values were recorded for EN32 (0.97), EN25 (0.96), EN24 (0.95), EN17 (0.95), Gz179 (0.95), and IET1444 (0.95). These current genotypes had the same trend as RDI. These findings are in harmony with Karimizadeh and Mohammadi (2011).

3.4 SCoT markers for drought tolerance

Forty-six SCoT markers were used to screen the status of drought tolerance genes in 10 mutants and the check varieties. Out of 46 markers, thirteen were found to be linked with drought tolerance genes (Table 8). The results of genotypic screening of the 14 rice genotypes for the presence or absence of rice drought tolerance genes linked to SCoT markers are shown in Table 8. The electrophoretic pattern for each SCoT marker linked to these studied genes is shown in Figure 1.

The genetic frequencies of the 13 major rice drought tolerance genes ranged from 64.3% to 78.6%, according to the data. The drought tolerance genes linked to SCoT markers are SCoT8, 1.674 bp, SCoT40, 3.615 bp, and SCoT44, 0.878 bp, and these are distributed in all tolerant genotypes with the check variety for drought tolerance IET1444 which shared the same bands. However, the sensitive genotypes did not have these SCoT markers. Whereas, SCoT11 (1.703 bp and 1.925 bp), SCoT40 (1.179 bp), and SCoT44 (0.709 bp) showed 71.4 % of gene frequency in all genotypes. Also, drought tolerance genes which are linked to SCoT1 (2.555 bp), SCoT6 (0.53 bp), SCoT12 (2.64 bp), SCoT23 (1.222 bp), SCoT29 (1.572 bp) and SCoT40 (0.946 bp) showed the lowest gene frequency across all genotypes. According to the data in Table 8, cultivars IET1444, Gz179 and mutants EN24 and EN28 are high tolerance to drought stress and possess thirteen genes that are linked to all selected SCoT markers in table 8. Although the mutant EN28 is drought tolerant, unfortunately, it has a relatively low yield. While in case of EN7

Table 8 Genotypic screening of 10 rice mutants and check cultivars for drought tolerance genes linked with SCoT markers

Primers	MS	Gz179	Gz178	EN32	EN28	EN27	EN26	EN25	EN24	EN17	EN14	EN46	EN7	SK107	IET	Gene Frequency
SCoT1	2.555	1	1	1	1	1	1	1	1	0	0	0	0	0	1	64.3
SCoT6	0.53	1	1	1	1	1	0	0	1	1	1	0	0	0	1	64.3
SCoT8	1.674	1	1	1	1	1	1	1	1	1	1	0	0	0	1	78.6
SCoT11	1.925	1	1	1	1	0	1	1	1	1	1	0	0	0	1	71.4
SCoT11	1.703	1	1	1	1	0	1	1	1	1	1	0	0	0	1	71.4
SCoT12	2.64	1	1	0	1	0	1	1	1	1	1	0	0	0	1	64.3
SCoT23	1.222	1	0	0	1	1	1	1	1	1	1	0	0	0	1	64.3
SCoT29	1.572	1	1	1	1	0	1	1	1	1	0	0	0	0	1	64.3
SCoT40	3.516	1	1	1	1	1	1	1	1	0	1	0	0	1	1	78.6
SCoT40	1.179	1	0	1	1	1	0	1	1	1	1	0	0	1	1	71.4
SCoT40	0.946	1	0	1	1	1	0	1	1	1	1	0	0	0	1	64.3
SCoT44	0.878	1	1	1	1	1	1	1	1	1	1	0	0	0	1	78.6
SCoT44	0.709	1	0	1	1	1	1	1	1	1	1	0	0	0	1	71.4
Total of tolerance genes		13	9	11	13	9	10	12	13	11	11	0	0	2	13	

The rice drought tolerance gene scored as the presence (1) and absence (0) of band linked to allele of SCoT markers; MS: Molecular size

and EN46 (highly susceptible) do not possess these genes as well as SK107 cultivar, which belongs to the same genus (Japonica) does not have most of the drought tolerance genes except for SCoT40 (1.179 bp and 3.516 bp) alleles. Therefore, the tolerance of this cultivar can be attributed to the SCoT40 gene.

Our results were similar to Patidar et al. (2022) and Xiong et al. (2011). They reported that the SCoT marker technique corresponds to functional genes and their correlating characters in rice. SCoT is a targeted marker with multilocus nature; besides, SCoT can generate more information correlated with biological traits and help in case of high genetic polymorphism. Evaluation of SCoT markers in diversity analysis and diagnostic finger printing has already been established in *Vigna unguiculata* (Igwe et al. 2017). Moreover, Gorji et al. (2011) presented that SCoT markers were more informative and compelling, followed by ISSRs and AFLP markers in fingerprinting of potato varieties. However, the simplicity and reproducibility of SCoT have been successfully applied to the assessment of genetic diversity and taxonomic study of Citrus (Han et al. 2011); rice (Collard and Mackill 2009); and barley (Aboulila and Mansour, 2017; Dora et al. 2017).

Out of 46 SCoT primers, 34 primers revealed a total of 377 PCR bands, while the remaining primers did not amplify bands among the studied genotypes. The total numbers of polymorphic bands were 373 (98.9%), while the remaining four were monomorphic (1%). The total number of polymorphic amplified bands by each primer ranged from 4 (primer SCoT 24) to 19 (primer SCoT 12) (Table 9), with 100% polymorphism for all primers except SCoT 44 and SCoT 2 (75 and 81.81, respectively). Also, four monomorphic bands were only amplified by primers SCoT 2 (2) and SCoT 8 (2), where the studied rice genotypes contained two sensitive ones for drought. The mediocre number of amplicons/primers was 11 (10.9 and 0.1 polymorphic and monomorphic bands, respectively). As well as, targeting regions in plant genes confers great importance on unique bands amplified with SCoT primers, especially in elite genotypes. The present investigation revealed fifty-three positive specific distinctive markers for high-yielding drought-tolerant rice genotypes, which suggests a role of such unique sequences in yield and drought tolerance (Table 9 and Figure 1). The amplification of unique SCoT bands in drought-tolerant genotypes was also recorded by Shaban et al. (2022). In harmony with our results, Emam et al. (2022) amplified unique SCoT bands with drought tolerance. Therefore, SCoT can be applied to differentiate between different drought stress tolerances according to markers associated with new alleles for this trait in given selected genotypes.

The UPGMA-based dendrogram (Figure 2) showed the genetic relationships among the rice genotypes for SCoT analysis. The dendrogram of the fourteen rice genotypes using the UPGMA procedure clustered these genotypes into three major groups by their

Table 9 The polymorphism for 14 rice genotypes using 34 SCoT markers

Primers	MB	PB	UB	TB	P (%)
SCoT1	0	11	0	11	100
SCoT2	2	9	0	11	81.81
SCoT5	0	16	1	16	100
SCoT6	0	10	2	10	100
SCoT7	0	12	1	12	100
SCoT8	0	13	2	13	100
SCoT9	0	6	1	6	100
SCoT10	0	7	1	7	100
SCoT11	0	15	2	15	100
SCoT12	0	19	2	19	100
SCoT13	0	9	2	9	100
SCoT14	0	8	5	8	100
SCoT16	0	9	1	9	100
SCoT19	0	12	3	12	100
SCoT20	0	13	4	13	100
SCoT21	0	10	0	10	100
SCoT22	0	9	0	9	100
SCoT23	0	13	1	13	100
SCoT24	0	4	0	4	100
SCoT26	0	12	5	12	100
SCoT28	0	13	3	13	100
SCoT29	0	13	2	13	100
SCoT31	0	11	1	11	100
SCoT32	0	9	2	9	100
SCoT33	0	11	2	11	100
SCoT34	0	14	3	14	100
SCoT35	0	14	2	14	100
SCoT36	0	9	2	9	100
SCoT37	0	7	1	7	100
SCoT39	0	13	1	13	100
SCoT40	0	13	0	13	100
SCoT42	0	12	1	12	100
SCoT44	2	6	0	8	75
SCoT46	0	11	0	11	100
Total	4	373	53	377	-----
Average	0.1	10.9	1.6	11.0	98.7

MB: Monomorphic bands, PB: Polymorphic bands, UB: Unique bands, TB: Total bands. P%: Polymorphism

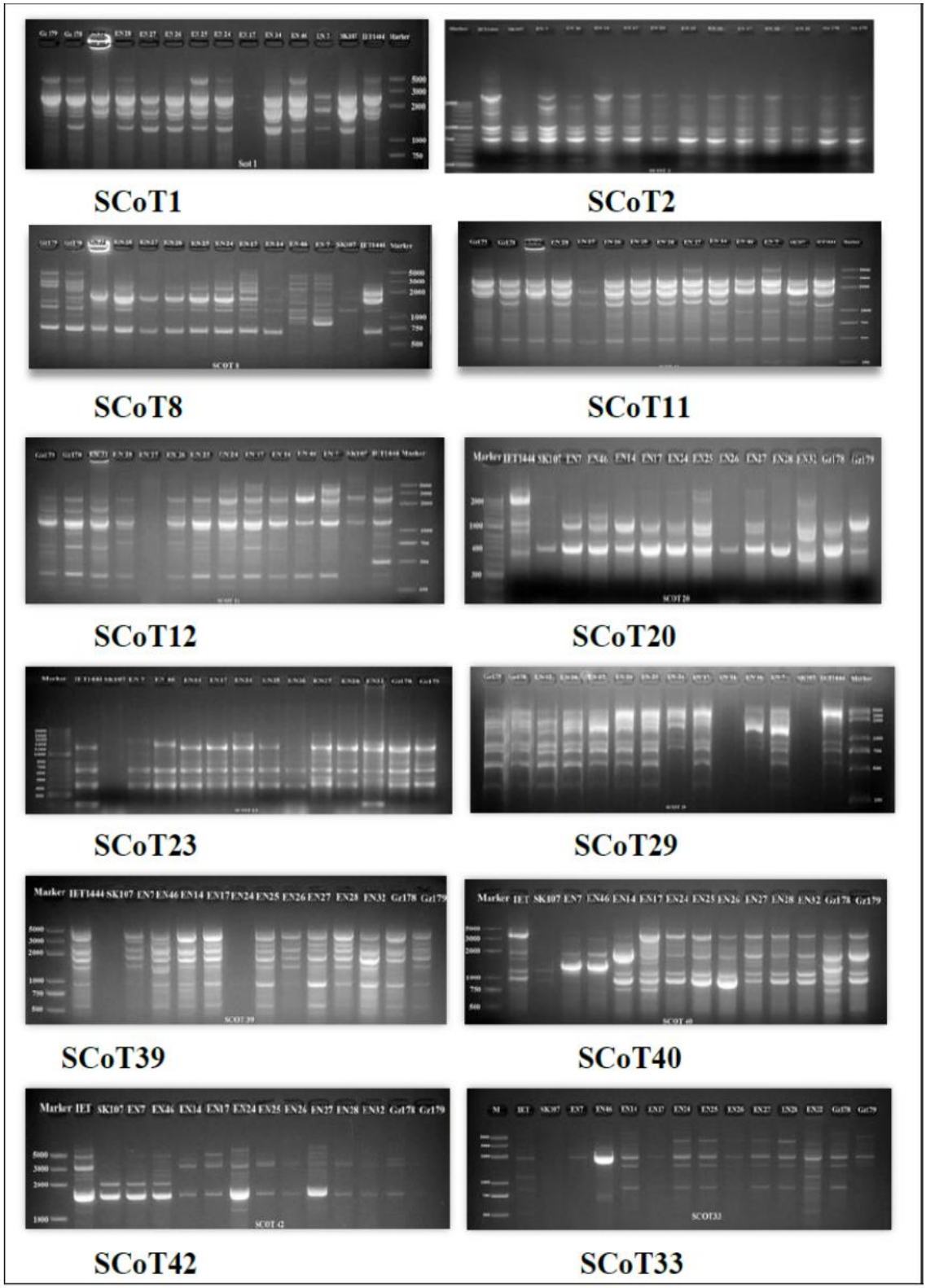

Figure1 SCoT profiles of 10 mutants of rice were produced using gamma radiation in M_8 generation and the check varieties using SCoT 1, SCoT 2, SCoT8, SCoT11, SCoT 12, SCoT 20, SCoT 23, SCoT29, SCoT33, SCoT 39, SCoT 40 and SCoT 42 primers

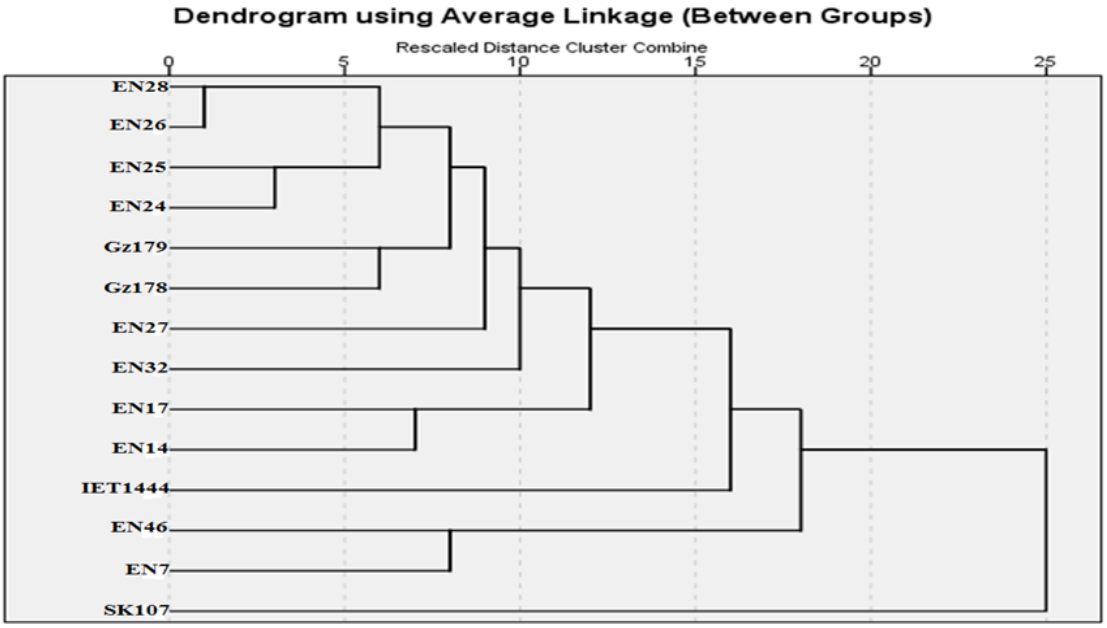

Figure 2 Dendrogram for Ten selected M_8 rice mutants constructed from SCoT data using (UPGMA) that computed according to Dice coefficients

Table 10 The similarity indices for all selected mutants of rice from M_8 and check varieties with SCoT primers

Genotypes	Gz179	Gz178	EN32	EN28	EN27	EN26	EN25	EN24	EN17	EN14	EN46	EN7	SK107
Gz178	68%												
EN32	59%	64%											
EN28	68%	71%	68%										
EN27	57%	58%	59%	70%									
EN26	61%	58%	57%	78%	61%								
EN25	62%	59%	54%	72%	59%	72%							
EN24	62%	61%	53%	70%	60%	58%	72%						
EN17	60%	56%	49%	59%	49%	53%	68%	62%					
EN14	56%	57%	49%	56%	46%	49%	55%	60%	64%				
EN46	41%	38%	35%	41%	32%	38%	45%	41%	50%	42%			
EN7	46%	36%	32%	44%	35%	45%	51%	46%	51%	41%	63%		
SK107	23%	17%	20%	27%	16%	27%	28%	29%	27%	32%	42%	39%	
IET	48%	49%	46%	47%	36%	40%	48%	48%	49%	55%	43%	42%	28%

reaction to drought tolerance response and the type of these mutants. Interestingly, the eleven drought-tolerant rice genotypes, i.e. EN28, EN26, EN25, EN24, Gz 179, Gz 178, EN27, EN32, EN17, EN14, and IET1444 belong to Indica- Japonica types were far from the other genotypes and were clustered together in one group (cluster I). A moderate drought-tolerant variety, Sk107 (Japonica type), was closer to the drought-tolerant genotypes (cluster II). The drought-sensitive rice genotypes EN46 and EN7 were very close and grouped in the same cluster (cluster III), representing the Japonica type. The cluster I genotypes showed a genetic similarity percentage ranging from 36% to 78% in the similarity indices (Table 10). On the other hand, cluster II included the Sk107 variety, which was isolated in a single group (a moderate japonica drought-tolerant variety). Drought-sensitive rice mutants EN46 and

EN7 (japonica) were separated into the same group (cluster III) with a genetic similarity percentage of 63%. The highest similarity value of 78% was recorded between the two mutants EN26 and EN28, followed by 72% among EN25 and EN24, EN26, and EN28; these values indicate that every two mutants with high similarity were closely related. While the lowest value recorded was 16% among genotypes SK107 and EN27; this indicates that these two genotypes were genetically distant types of genotypes. These results confirm the capability of SCoT as an excellent marker to establish the genetic relationships between various cultivars and obtain new specific clustering (Xiong et al. 2011; Etminan et al. 2016).

Conclusions

Mutation breeding is a beneficial method to create new rice genotypes that are resistant to the effects of drought. Ten mutants were evaluated under three irrigation intervals (irrigation every 4, 8, and 12 days) for yield and yield-related traits. Moreover, these mutants were evaluated using different drought-tolerant indices. According to the previous methods, the obtained results exhibited seven high-tillering drought-tolerant mutants with high yields compared with check varieties under irrigation conditions. The STI, MP, YI, and GMP indices present that the mutant EN25 showed the highest ability under drought stress followed by EN27 in comparison with IET444 (DT check variety). These mutants will save more than 30% of irrigation water with maintaining high productivity and can be used as sources of tolerance genes in breeding programs. Furthermore, using DNA markers linked to the tolerance genes is a powerful tool in identifying and screening these specific genes between rice genotypes. Moreover, SCoT markers in this study successfully evaluated the genetic relationships among the 14 rice genotypes. All PCR primers generated a high level of polymorphism 100%, except SCoT2 and SCoT44. The 14 genotypes were clustered into three clusters using the UPGMA dendrogram based on their tolerance to drought stress. SCoT showed that these seven mutants share 13 of the same bands with IET444 (check variety). The results of the present study will be useful for releasing new drought-tolerant rice cultivars.

Conflict of interest

The authors declare no conflict of interest

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