Journal of Applied Botany and Food Quality 97, 140 - 149 (2024), DOI:10.5073/JABFQ.2024.097.017

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Physiological characterization and assessment of genetic variability, yield, and quality properties of gamma-ray-induced salinity tolerant soybean (*Glycine max* (L.) Merrill) mutants

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(Submitted: January 22, 2024; Accepted: August 6, 2024)

Summary

Soybean is an important industrial oilseed plant. As a relatively fast, flexible, cheap, and viable method, mutation breeding, which induces significant random genetic variations, is a widely used method in crop science. In the present study, we investigated physiological parameters, genetic variability, yield, and quality properties of salinity-tolerant mutant plants derived from Ataem-7 and S04-05 soybean varieties by Cs-137 gamma radiation-induced mutations. The SM4 and SM3 mutants exhibited a greater genetic distance than all other salinity tolerant mutants did. SM3 mutant presented 16.8% lower lipid peroxidation under salinity stress. The most significant photosynthetic pigment increase was detected for chlorophyll b in SM4 and SM3 mutants, with values of 1.88 and 2.07-fold, respectively. The SM3 mutant exhibited the highest yield, at 437.6 kg/ha in the M_3 generation, while AM1 presented the highest yield in the M_4 generation. The AM1 mutant also had the highest pod count by 122.2 per plant. In the AM1 mutant, the photosynthetic pigment increase was 16.69% for chlorophyll a, 37.9% for chlorophyll b and 22.9% for total chlorophyll. These results provide a basis for future investigations in soybean mutation breeding studies for salinity stress tolerance, and also indicate the effectiveness of mutation breeding methods in agricultural breeding programs.

Keywords: *Glycine max* (L.) Merr; salinity tolerance; mutation breeding; fatty acids

Introduction

Soybean, *Glycine max* (L.) Merrill, is a legume member of the Fabaceae family, in which roots form nodules to house the nitrogen fixing bacteria exclusive to this family members (WAGNER, 2011). Among leguminous seed crops, it is one of the most important oil crop plants. The United States of America, Brazil, and Argentina are currently the leading soybean producers in the world accounting for more than 82% of approximately 400 million metric tons of total soybean production worldwide (FAO, 2022). Soybean, which is extremely beneficial for food, feed, and industrial raw material purposes due to its high protein content, high levels of essential fatty acids, various vitamins, and minerals, is produced under relatively wide climate, soil, and growth conditions.

In the verge of expected harmful natural hazards caused by global warming, salinity, which is defined as the accumulation of soluble salts on the upper layer of the soil over 4 dS m^{-1} electrical conductivity (EC) value, is the secondary abiotic stress factor that generally comes along with drought. Irrigation applications in arid lands lead to the solid accumulation of salts after the evaporation of water. Except increasing global temperatures, wrong irrigation and fertilization approaches, excessive precipitation, lack of drainage, geological and climatic properties contribute to the present salinization levels. 7% of the world lands and 20% of irrigated agricultural lands have already exceed the tolerable salinity limits for many crop plants (REDDY et al., 2017).

Low genetic variation in cleistogamous soybean plants requires intensive breeding programs for desired trait development. In nature, the magnitude of genetic variations depends on available gene pool and inter-relations within populations. Improving soybean for desired traits through classical approaches is challenging since it has limited gene pool and inter-relations within populations. Therefore, inducing genetic variations in the genome is the pre-requisite step in soybean breeding programs. Mutation breeding offered great advantages to the plant breeders for inducing genetic variations in various agronomic traits during the last decades (JEGADEESAN and PUNNIYAMOORTHY, 2023). The combination of agricultural approaches with mutagens allows relatively fast, flexible, cheap, and viable crop improvement. Since 2000, there have been 46 registered soybean mutants in Mutant Variety Database (MVD) of joint initiation of International Atomic Energy Agency (IAEA) and Food and Agriculture Organization of United Nations (FAO) (IAEA, 2022). Early maturation, resistance to various biotic and abiotic stresses, high yield, super nodulation, higher nitrogen fixation and higher protein content are among the most widely improved traits through mutation breeding in soybean.

In the present study, gamma-ray-induced salinity tolerant soybean mutants, which were derived from S04-05 and Ataem-7 varieties were obtained and maintained for four generations to sustain genetic stability after mutagenesis. Salinity tolerances of original varieties and M_4 generation mutants were evaluated through growth parameters of average plant height and weight and biochemical parameters of chlorophyll content, proline accumulation, lipid peroxidation and total carbohydrate content. Yield and quality analysis of the mutant plants were also performed comparing to their original varieties over parameters of average plant weight (g), first pod heights (cm), pod counts, seed yield (kg/ha), 1000 seeds weights (g), oil content (%), protein content (%), fatty acid content (%), while polymorphism between salinity tolerant mutants and their original varieties was determined by using RAPD markers.

Materials and methods

Determination of optimal irradiation dose

Ataem-7 and S04-05 soybean seeds were obtained from Black Sea Agricultural Research Institute (Samsun, TURKEY). Relative water content of the seeds was adjusted to 12% before the irradiation experiments. Sixty seeds were irradiated with a Cs-137 gamma radiation source in the IBL 437C irradiation facility belonging to Our Leukemia Children Foundation. The dose rate of the irradiation facility was 6.248 Gy/min on experimentation day. Therefore, soybean seeds of experimental groups were irradiated by 0, 100, 150, 200, 250, 300, 400, 500 Gy gamma radiation doses in durations as 0, 16.00, 24.00,

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32.01, 40.01, 48.02, 64.02, 80.03 minutes, respectively. The control group was carried out together with all experimental groups except the irradiation process. Irradiated seeds were sown in plastic boxes. Plants were grown under controlled conditions with 16/8 hours of light/dark period and 26/18 °C. Plantlets were watered with Hoagland nutrient solution (HOAGLAND and ARNON, 1950). The GR_{30} and GR_{50} doses, which decrease the growth by 30 and 50%, respectively, were determined according to the average seedling heights of 14-days-old plantlets. Each experimental condition was designed in three replicates.

Determination of salinity tolerance of soybean varieties

Sixty seeds belonging to both varieties were germinated in 9×5-well viols containing perlite as filling (20 seeds per viol in three replicates). Plantlets were watered with Hoagland nutrient solution (HOAGLAND and ARNON, 1950) for 14 days. After the 14th day, salinity treatment was initiated by addition of 30, 60, 90, 120 and 150 mM NaCl to the nutrient solution for 7 days, continuously. Salinity tolerance capacities were determined according to the parameters of photosynthetic pigment content, thiobarbituric acid reactive substances (TBARS) content and proline accumulation in gamma-ray-induced mutants. Each experimental condition was designed in three replicates including the control group.

Induced mutagenesis and field experiments

Based on the determined GR_{30} and GR_{50} doses, 12,000 seeds (1200 for each group) belonging to Ataem-7 and S04-05 soybean varieties were irradiated by 100, 150, 200, and 250 Gy gamma rays using a Cs-137 gamma radiation source for mutagenesis. Seeds were planted in the experimental fields of the Black Sea Agricultural Research Institute. Parameters of germination rate, seedling survival (on the 28th day), days to 50% flowering were determined for M_1 generation mutant plants.

***In vivo* salinity tolerance screening in M_2 and M_3 generations**

The M_1 generation seeds of soybean mutants were sown into perlite and irrigated with Hoagland solution containing 90 mM NaCl for 7 days for *in vivo* salinity tolerance screening studies. The seedlings were kept under controlled conditions (16 h/8 h light/dark period at 26/18 °C). The mutant plants, which present normal growth as control groups without stress indications, were selected and transferred into perlite filled pots and irrigated with Hoagland solution on a daily basis. The M_2 generations seeds of selected salt tolerant soybean mutants were harvested, and the screening procedure was also repeated for M_3 generation.

***In vitro* salinity tolerance screening in M_2 and M_3 generations**

Salinity tolerance of the M_1 generation mutants was also confirmed through further *in vitro* selections. Leaf explants of *in vivo* selected M_2 mutants and the control plants were transplanted onto the petri dishes containing callus induction medium including Gamborg's B5 inorganics and vitamins, 30 g/L sucrose, 9 g/L agar, 1 g/L L-glutamine, 40 mg/L adenine sulphate, 1 mg/L 2,4-D and 90 mM NaCl. For the negative control, the leaf explants of control plants were cultured on a callus medium without NaCl. For a positive control, the leaf explants of the control plants were cultured on callus medium containing 90 mM NaCl. The callus cultures were maintained in a growth chamber under controlled conditions (16 h/8 h light/dark period at 26/18 °C). *In vivo* selected salt tolerant soybean mutants were confirmed according to the callus growth with respect to the positive and negative controls. The screening procedure was also repeated for M_3 generation mutants.

Field conditions for M_4 generation

90 mM NaCl tolerant 13 different (5 of mutants derived from Ataem-7 and 8 mutants derived from S04-05 variety) M_3 generation soybean mutants were subjected to field trials. Black Sea Agricultural Research Institute trial fields were at 4 meters altitude with basic (pH 7.8) loamy clay properties containing mild organic matter content. Average field temperature and total precipitation values were 20.5 °C and 218.6 mm, respectively. Average sunlight exposure was 7.4 hours/day. Trials were initiated in random block pattern with three replicates. Ataem-7 and S04-05 were planted as control groups. Replicates were planted in three lines in 5 × 0.7 m^2 parcels. There were 45 plants per m^2 area. Standard soybean varieties were planted on the outside border of the parcels to prevent outside effects. The drip irrigation method was applied for watering. Manual weeding of the field was performed twice when the seedlings reached the height of 20 - 25 cm and 40 - 60 cm. There was no indication of disease or pest in the trial field. Seeds of all experimental groups were harvested separately. 30 random plants of each group were used for yield and quality analysis.

Salinity tolerance screening in M_4 generation mutants and original varieties

Sixty seeds of each mutant were germinated in 9×5-well viols containing perlite as filling (20 seeds per viol in three replicates). Plantlets were watered with Hoagland nutrient solution (HOAGLAND and ARNON, 1950) for 14 days. After 14th day salinity stress was initiated by addition of 90 mM NaCl to the solution for 7 days. Salinity tolerance was evaluated through growth parameters as average plant height and weight and biochemical parameters as chlorophyll content, proline accumulation, lipid peroxidation and total carbohydrate content.

Biochemical analysis

Photosynthetic pigment contents

Effects of salinity stress on photosynthetic pigment contents of soybean plants were evaluated as described by ARNON (1949). 0.1 g leaf tissue was grounded to fine powder by using liquid nitrogen. Pigments were extracted by adding 80% cold acetone. Extracts were filtered through Whatman No.2 filter paper. Samples were measured at 470, 645 and 663 nm, spectrophotometrically. Photosynthetic pigment contents were calculated according to the equations below:

$$\text{(Chlorophyll a (Chll a) Concentration (mg / g} \times \text{FW)} = 11.24 \times A663 - 2.04 \times A645)$$

$$\text{(Chlorophyll (Chll b) Concentration (mg / g} \times \text{FW)} = 20.13 \times A645 - 4.19 \times A663)$$

$$\text{(Total Chlorophyll (mg / g} \times \text{FW)} = 7.05 \times A663 + 13.09 \times A645)$$

$$\text{(Carotenoid (mg / g} \times \text{FW)} = (1000 \times A470 - 1.9 \times \text{Chll a} - 63.14 \times \text{Chll b})/214)$$

Lipid peroxidation

Effects of salinity stress on lipid peroxidation of soybean plants were evaluated by the method of STEWART and BEWLEY (1980). Leaf samples were grounded in liquid nitrogen, and 1 mL of extraction medium containing 0.5% (w/v) thiobarbituric acid and 20% (w/v) trichloric acid was added. The mixture was heated for 1 h at 100 ºC. Samples were cooled to 4 ºC to stop reactions. After centrifugation for 10 min at 3,000 × g, the absorbances of the supernatants were measured at 532 and 600 nm. The standard calibration curve was prepared by using 1, 2, 4, 8, 16, 32, and 64 µg proline dilutions. The extinction coefficient for MDA was 155 $mM^{-1} \cdot cm^{-1}$. The results were expressed as µmol $MDA \cdot g^{-1} \cdot FW$ (RAO and SRESTY, 2000).

Total carbohydrate content

The effects of salinity stress on total carbohydrate content of soybean plants were evaluated by Dubois method (DUBOIS et al., 1956). 0.1 g of leaf tissues were grounded in distilled water and heated to 100 °C for 3 h. Samples were centrifuged at 10,000 × g for 15 min. One mL of the supernatant was mixed with 1 mL of phenol and 5 mL of 96% sulfuric acid. The mixture was kept at room temperature for 10 min then incubated in 30 °C water. The chromophore was measured at 490 nm. Total carbohydrate content was calculated by using the standard calibration curve, which is prepared by 0.02, 0.04, 0.06, and 0.08 mg/mL sucrose dilutions.

Proline accumulation

Proline accumulation was determined according to the method of Bates (BATES et al., 1973). Extracts were prepared in 3% sulphosalicylic acid. Filtered extracts were boiled at 100 °C for 1 h following the addition of acid ninhydrin reagent (ninhydrin and glacial acetic acid). The reaction was stopped in an ice bath and fractions were separated by using toluene. Absorbance values were measured at 520 nm wavelength. Proline levels were calculated by using a standard calibration curve in unit of μmol proline/g.FW.

Yield tests and quality analysis of mutant plants

Yield and quality analyses of the mutant plants comparing to their original varieties were performed in Black Sea Agricultural Research Institute according to the random trial blocks design. Under field conditions, each mutant and original variety were planted in 5-meter-long lines in two rows for replicate. 70 cm distance was applied between the lines. 45 plants were planted in each 45 m^2 area. Random soybean varieties were planted around the test field to prevent environmental border effects. Each mutant and original variety were harvested separately as bulk. Average plant weights (cm), first pod heights (cm), pod counts, seed yield (kg/ha), 1000 seeds weights (g), oil contents (%), protein contents (%), and fatty acid contents (%) were measured. Gas chromatography with FID detector was used to determine palmitic, oleic, linoleic, and linolenic acid contents.

Polymorphism analysis

Polymorphism between salinity tolerant mutants and their original varieties was determined by using RAPD markers (Tab. S1). Genomic DNA was extracted by CTAB (cetyltrimethylammonium bromide) method by using 100 mg of leaf samples as previously described by MERIÇ et al. (2023). Leaf tissue was grounded into fine powder by using liquid nitrogen. 300 μL of CTAB solution was added to homogenates. After gently shaking samples, additional 500 μL of CTAB solution was added. Samples were incubated at 65 °C for 90 min following the addition of 20 μL Proteinase K (Sigma, P2308). At the end of incubation, 20 μL of 10 mg/mL RNase A (Sigma, R4642) was added to the samples and samples were incubated at 65 °C for 10 min. Samples were centrifuged at 16,000 × g for 10 min. 500 μL of supernatant was transferred to the fresh tube and 500 μL of chloroform was added. Samples were mixed and centrifuged at 16,000 × g for 5 min. Supernatants were transferred to fresh tubes and 2 volumes of CTAB precipitation solution was added. Samples were mixed by using a micropipette, gently. Then, samples were incubated at room temperature for 1 h. Samples were centrifuged at 16,000 × g for 5 min and supernatants were discarded. 350 μL of 1.2 M NaCl solution was added to the pellets. 350 μL of chloroform was added on dissolved pellets. Mixed samples were centrifuged at 16,000 × g for 10 min. Supernatants were transferred to fresh tubes and 0.6 volume of isopropanol (Sigma, I9516) was added. Samples were centrifuged at 16,000 × g for 10 min. 500 μL of 70% ethanol (Sigma, E7033) was added to supernatants. Mixed samples were centrifuged at 16,000 × g for 10 min. Pellets were dissolved in 30 μL of sterilized distilled water. Reactions were run by using a thermal cycler in a total volume of 25 μL, comprised of 17 μL sterile distilled water, 5 μL of 5X PCR Buffer (1X/μL) containing 1 mM dNTP and 3 mM $MgCl_2$, 1 μL of primer (0.4 μM/μL), 1 μL of 30 ng/μL template genomic DNA (1.2 ng/μL) and 1 μL of DNA polymerase enzyme (0.04 U/μL) under the following conditions: 1 min at 95 °C (denaturation), followed by 35 repetitions of 15 s at 95 °C (denaturation), 15 s at the values presented in Tab. S1 for annealing and, 3 min at 72 °C (extension). The amplified bands for each sample per random primers were scored as "0" or "1" scores according to the absence or presence of a band. Similarity coefficients were calculated according to Jaccard similarity index. Sequential Agglomerative Hierarchical Non-Overlapping (SAHN) clustering was applied using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA). Dendrograms were plotted using NTSYS-PC Ver. 2.1 software.

Statistical analysis

The experiments were repeated three times, and each data point was the mean of three replicates. Statistical evaluation of the means was performed by using one-way ANOVA analysis and statistically meaningful data were compared using Student-Newman-Keuls test performed by using GraphPad Prism version 8.0.1 for Windows statistics software.

Results

Determination of optimal irradiation dose and salinity tolerance for soybean varieties

GR_{30} doses were calculated as 138 Gy and 265 Gy, while the GR_{50} doses were calculated as 225 Gy and 385 Gy for S04-05 and Ataem-7 varieties, respectively (Fig. 1). A regression analysis was performed between seedling heights and gamma radiation doses and the R^2 values were determined as 0.9496 and 0.971 for S04-05 and Ataem-7 varieties, respectively. Based on these results, 100, 150, 200, and 250 Gy gamma radiation doses were selected for mutation induction.

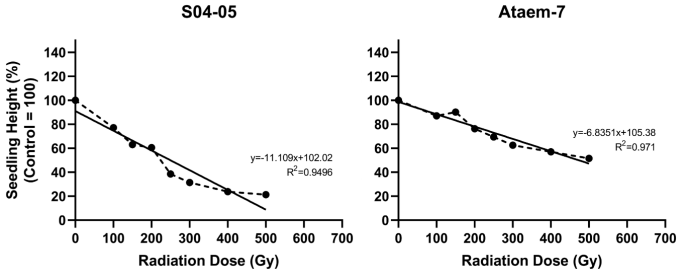

Fig. 1: GR_{30} and GR_{50} doses, which decrease growth rate 30 and 50% of S04-05 and Ataem-7 soybean varieties. GR_{30} doses were calculated as 138 Gy and 265 Gy, while the GR_{50} doses were calculated as 225 Gy and 385 Gy for S04-05 and Ataem-7 varieties, respectively.

Determination of salinity tolerance of soybean varieties

Optimal salinity concentration was determined according to the parameters of photosynthetic pigment content, TBARS content and proline accumulation for salinity tolerance screening of the gamma-ray-induced mutants (Fig. 2).

Chlorophyll a content of Ataem variety presented statistically significant decreases between all experimental groups except 30 and 60 mM compared to the control. The decrease was 6.14, 13.3, 23.8, 30.2 and 47.1 %, respectively. Chlorophyll b content of Ataem variety presented statistically significant decreases only in 30-, 120- and 150-mM

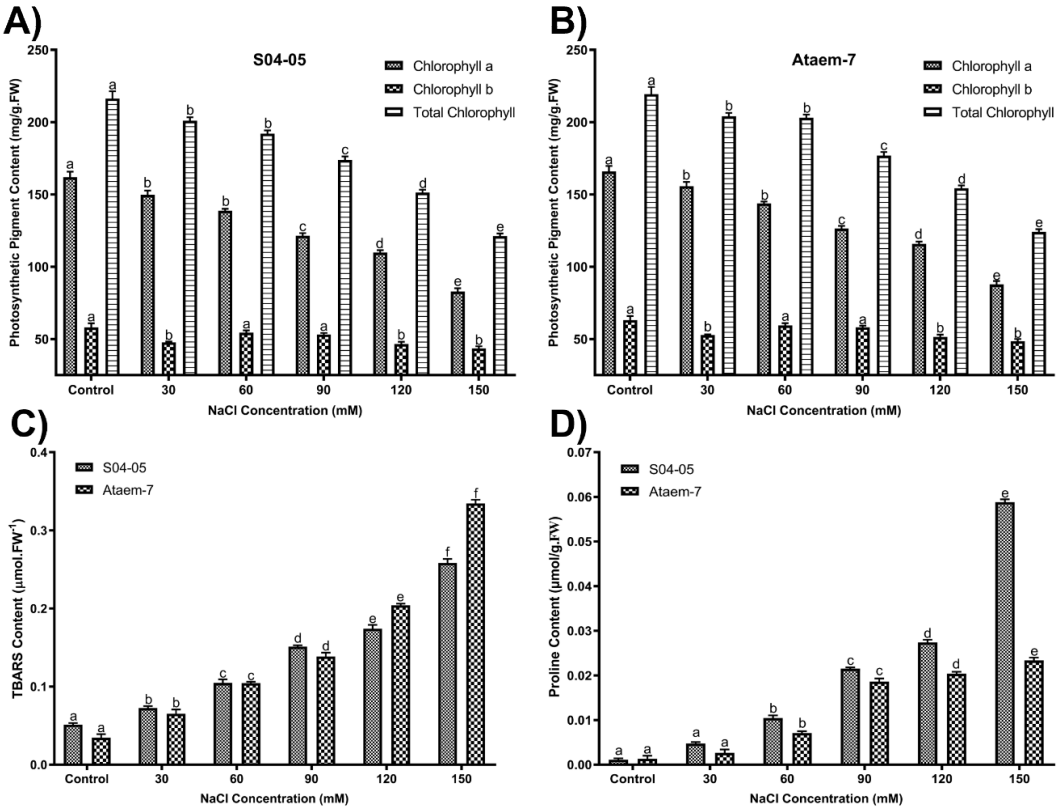

Fig 2: Effects of salinity on parameters of A) and B) photosynthetic pigment contents, C) TBARS content and D) proline accumulation for S0405 and Ataem-7 soybean varieties [Different letters indicate statistically significant differences ($P \leq 0.05$)].

experimental groups. The decrease was 16.4, 18.3 and 23.1%, respectively. Similarly, chlorophyll a content of S04-05 variety presented statistically significant decreases between all experimental groups except 30 and 60 mM compared to the control. The decrease was 7.5, 14.3, 25.0, 32.2, and 48.9%, respectively. Chlorophyll b content of S04-05 variety presented statistically significant decreases only in 30-, 120- and 150-mM experimental groups. The decrease was 17.7, 19.9, and 25.0%, respectively.

Proline accumulation was significantly increased in all NaCl treatments except 30 mM for both varieties. The increase was 9.3-fold for 60 mM, 19.3-fold for 90 mM, 24.25-fold for 120 mM and 52.04-fold for 150 mM in S04-05 variety. In Ataem-07 variety, the increase was 5.26-fold for 60 mM, 13.78-fold for 90 mM, 15.11-fold for 120 mM, and 17.26-fold for 150 mM.

TBARS content, which represents oxidative damage level in lipid membranes, was significantly increased in all applied NaCl concentrations. The increase was 1.42-fold for 30 mM, 2.04-fold for 60 mM, 2.94-fold for 90 mM, 3.39-fold for 120 mM and 5.03-fold for 150 mM in S04-05 variety. In Ataem-07 variety, the increase was 1.89-fold for 30 mM, 3.02-fold for 60 mM, 4.01-fold for 90 mM, 5.9-fold for 120 mM and 9.68-fold for 150 mM.

Since all three parameters were affected by all NaCl treatments, 90 mM was selected for further salinity tolerance screening of mutant plants due to the initiation of more dramatic alteration in this concentration.

Induced mutagenesis and M_1 generation field experiments

M_1 generation of mutants was evaluated through germination rates, seedling survival rates on the 28th day of planting, days to 50% flowering, and fertility (Tab. 1). The germination rates were reduced with respect to increasing gamma radiation doses. Irradiated Ataem-7 soybean variety presented higher germination rate by 65.08% at 250 Gy gamma radiation treatment comparing to the rate of S04-05 by 41.6%. On the 28th day, seedling survival rates were 80.21% and 76.05% for S04-05 and Ataem-7, respectively. The rates were reduced to 41.58% and 56.75% in 250 Gy irradiated plants, respectively. Days until 50% flowering of the seedlings were also affected with increasing gamma

Tab. 1: Field trial results of germination rate (%), seedling survival in 28th day, days to 50% flowering and fertility (%) in M_1 generation mutants.

	Control		100 Gy		150 Gy		200 Gy		250 Gy	
	S04-05	Ataem-7	S04-05	Ataem-7	S04-05	Ataem-7	S04-05	Ataem-7	S04-05	Ataem-7
Planted Seed Count	1200	1200	1200	1200	1200	1200	1200	1200	1200	1200
Germination Rate (%)	80.22	76.05	74.33	75.33	64.58	70.17	55.31	65.09	41.6	65.08
Seedling Survival in 28th Day (%)	80.21	76.04	74.33	75.33	64.58	70.17	55.31	65.08	41.58	56.75
Days to 50% Flowering	56	59	63-64	69-70	63-64	69-70	66-68	77-78	73-74	82-83
Fertility (%)	46.04	50.63	52.33	39.42	46.17	39.25	25.75	29.83	16.33	21.42

radiation. The flowering days were determined as 56 and 59 for S04-05 and Ataem-7 control plants, respectively. The flowering time was extended to 73 - 74 days and 82 - 83 days in 250 Gy irradiated groups, respectively. Fertility rates were also gradually decreased in accordance with increasing gamma radiation doses in both varieties.

Field results of M_4 generation mutants

In all trial blocks, mutant plants presented flowering and generated legumes. 30 plants from each trial parcel were randomly selected for statistical analysis and the rest of the plants were harvested to obtain M_4 generation seeds. Seed counts of each mutant were presented in Tab. 2.

Tab. 2: Harvested seed counts of mutant M_4 generation plants.

Mutant Code	Seed Count
SM1	1850
SM2	1482
SM3	3547
SM4	3100
SM5	1897
AM1	2000

Growth parameters in salinity treated M_4 generation mutants and original varieties

Growth parameters of M_4 generation mutants and their original varieties were evaluated through average plant heights and weights under salinity stress conditions. (Fig. 3). Salinity stress reduced average plant heights in Ataem-7 variety and its AM1 mutant, significantly. Average plant heights reduced 43.51% in Ataem-7, while they reduced 46.62% in AM1 mutant. Likewise, S04-05 variety and all its mutants presented decreased average fresh height. Average plant heights were reduced 33.04% in salt treated S04-05 variety, while they reduced 42.68% in SM1 mutant, 44.91% in SM2 mutant, 64.97% in SM3 mutant, 60.51% in SM4 mutant and 54.46% in SM5 mutant. Salinity stress also reduced average fresh weight in all experimental groups significantly. Average plant weights were reduced 51.14% in Ataem-7, while they reduced 47.73% in AM1 mutant. Likewise, S04-05 variety and all its mutants presented decreased average fresh weight. Average plant heights were reduced 40.0% in salt treated S04-05 variety, while they reduced 60.95% in SM1 mutant, 38.57% in SM2 mutant, 60.0% in SM3 mutant, 60.94% in SM4 mutant and 55.24% in SM5 mutant.

Biochemical analysis

Photosynthetic pigment contents

Carotenoid content of Ataem-7 variety was significantly increased 28.75% after salinity stress, while changes in chlorophyll a and total chlorophyll contents were not significant. Chlorophyll b content decreased 27.54% (Fig. 4). However, AM1 mutant presented significantly increased photosynthetic pigment content after the stress treatment, while changes in carotenoid were insignificant. In AM1 mutant, the increase was 16.69% for chlorophyll a, 37.85% for chlorophyll b and 22.86% for total chlorophyll. On the other hand, photosynthetic pigment contents of S04-05 variety and all its mutants were significantly increased after salinity stress except SM1 mutant, while carotenoid contents were significantly decreased in all mutants. The most significant increase was detected for chlorophyll b of SM4 and SM3 mutants which were calculated as 1.88 and 2.07-fold.

Lipid peroxidation

TBARS contents of the mutants and their original varieties were evaluated as the indicator of oxidative stress related lipid peroxidation (Fig. 5). Among salinity stress treated mutants, changes were not significant, except for the SM3 and SM1 mutants. SM3 and SM1 mutants presented 16.76 and 20.67% lower lipid peroxidation.

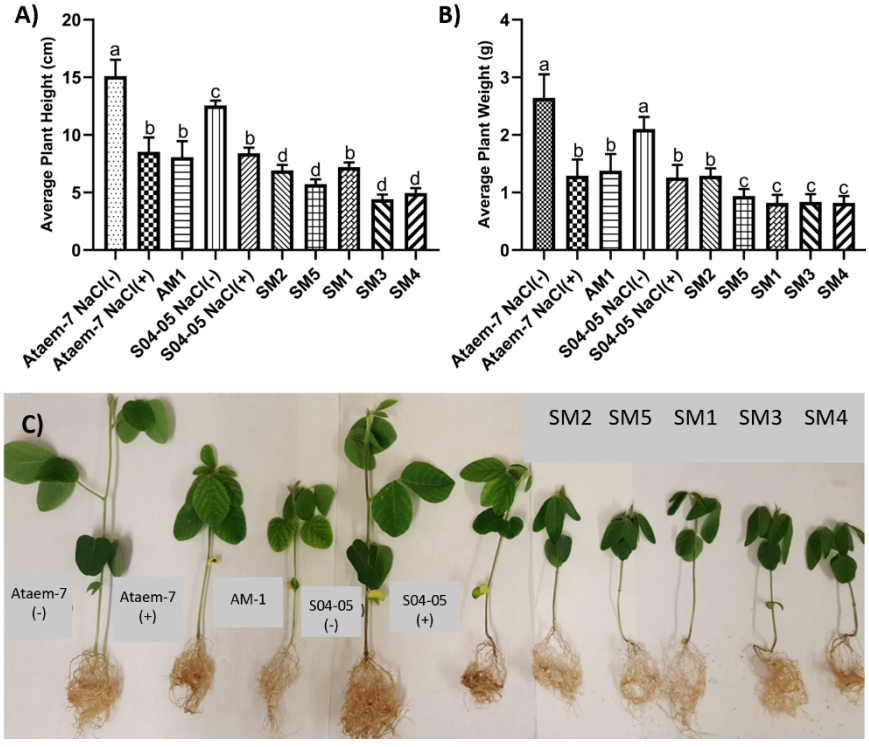

Fig. 3: Growth parameters of A) average plant height B) plant fresh weights of M_4 generation mutants and their original varieties [Different letters indicate statistically significant differences ($P \leq 0.05$)]. C) Representative image of mutants and original varieties.

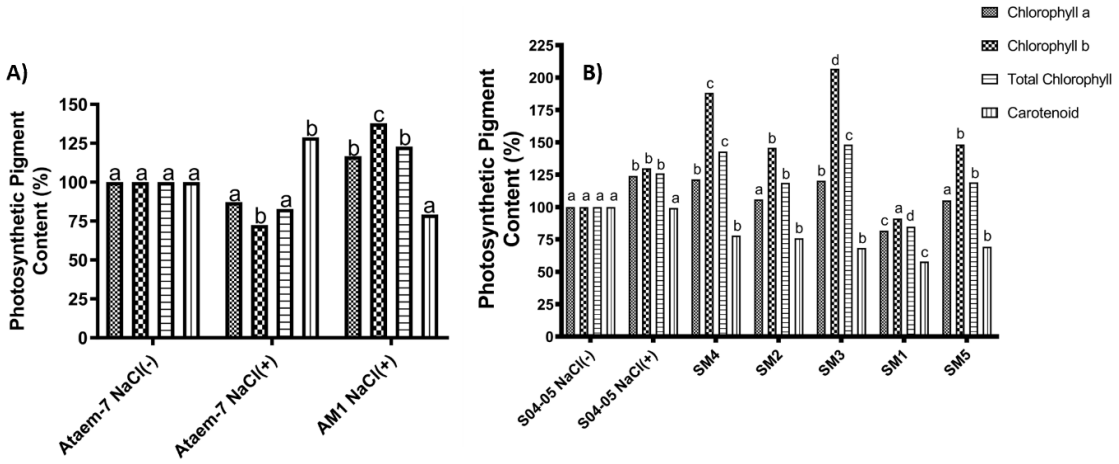

Fig. 4: Photosynthetic pigment contents of A) Ataem-7 variety and mutant derived from Ataem-7, B) S04-05 and mutants derived from S04-05 variety [Different letters indicate statistically significant differences ($P \leq 0.05$)].

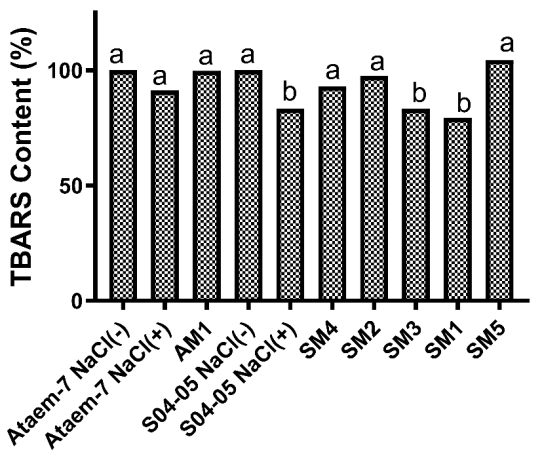

Fig. 5: Effects of 90 mM NaCl on lipid peroxidation of S04-05, Ataem-7 and mutants derived from both varieties [Different letters indicate statistically significant differences ($P \leq 0.05$)].

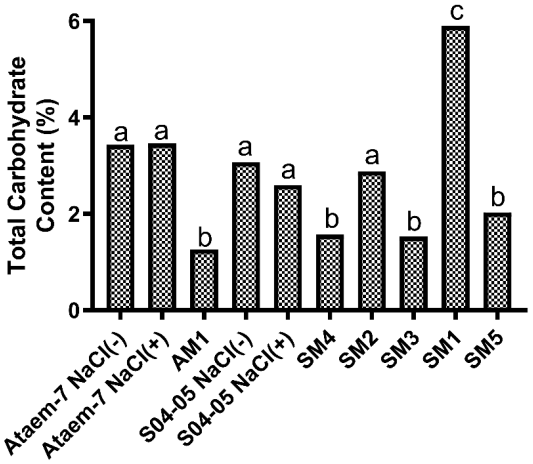

Fig. 6: Effects of 90 mM NaCl on total carbohydrate contents of S04-05, Ataem-7 and mutants derived from both varieties [Different letters indicate statistically significant differences ($P \leq 0.05$)].

Total carbohydrate content

Total carbohydrate content of the mutants was significantly decreased after salinity stress treatment (Fig. 6). However, SM1 mutant significantly increased carbohydrate level 2.27-fold, while SM2 was not statistically altered. Likewise, AM1 mutant of Ataem-7 variety decreased total carbohydrate contents significantly 63.56%.

Proline accumulation

Among salinity stress treated mutants and their original varieties, only S04-05 and SM5 mutant presented significantly increased proline accumulation (Fig. 7). The increase was 15.37 and 16.64%, respectively.

Polymorphism analysis

Genetic distance between untreated and salinity treated S04-05 and Ataem-7 varieties and their mutants were determined by polymorphism rates resulted from 17 random RAPD primers. Monomorphic or polymorphic bands were obtained for all 17 primers in salinity treated groups (Fig. S1 - S17). In salinity treatment, 61 of totally obtained 196 bands were polymorphic. The polymorphism rate was calculated as 31.12%. The UPGMA dendrogram was presented in Fig. 8.

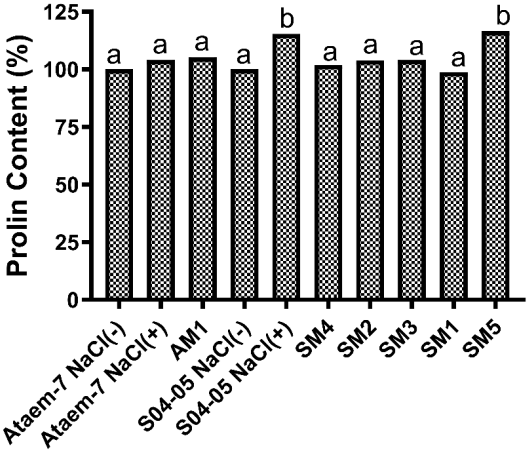

Fig. 7: Effects of 90 mM NaCl on proline accumulation of S04-05, Ataem-7 and mutants derived from both varieties [Different letters indicate statistically significant differences ($P \leq 0.05$)].

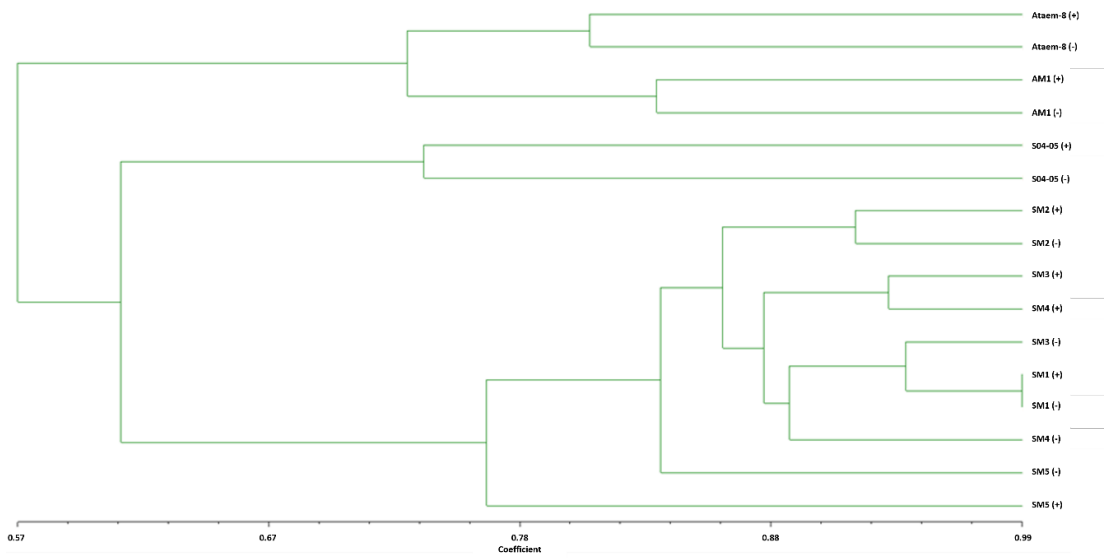

Fig. 8: The UPGMA dendrogram presenting genetic distance among all salinity tolerant mutants and original varieties.

According to the dendrogram, salinity treated and untreated samples of SM4 and SM3 mutants presented higher genetic distance among all other salinity tolerant mutants.

Yield tests and quality analysis of mutant plants

In M_3 generation field trials, 1000 seed weights, oil and protein contents presented significant difference comparing to their original and other standard soybean varieties (P<0.01) (Tab. 3). Yield (kg/ha) and plant height values were also significantly altered by the value of P<0.05. Changes in first pod height and pod count values were not statistically significant. SM3 mutant presented the highest yield by 437.6 kg/ha, while SM2-1 mutant had the lowest by 293.8 kg/da. Average plant heights of experimental groups were found statistically significant, and the values were between range of 132.5 and 89.2 cm. Ranges of oil and protein contents were between 21.1 - 23.39 and 39.84 - 42.63, respectively.

In M_4 field trials, 1000 seed weights, average plant heights, and oil contents were found statistically significant (P<0.01) (Tab. 3). Yield, protein content and pod count also presented significant alterations by value of P<0.05. Changes in values of first pod heights were insignificant. AM1 and SM4 mutants present the highest and the lowest yield values, respectively. AM1 mutant also had the highest pod count by 122.2/plant. Average plant heights of experimental groups were found statistically significant, and the values were between range of 156.3 and 122.7 cm. Ranges of oil and protein contents were between 20.77 - 23.14 and 39.94 - 43.62, respectively.

In 2017 field trials, SM4 mutant presented the highest oleic acid content (Tab. 4). Ataem-7 and SM2 had the highest linoleic acid content. Palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid contents were between ranges of 9.74 - 11.78, 3.68 - 4.21, 21.8 - 30.78, 47.53 - 53.64 and 6.13 - 7.92 %.

Tab 3: Field trial yield and quality analysis results belonging to years of M_3 and M_4 generations.

Sample	Yield (kg/da)		First Pod Height (cm)		Pod Count		Plant Height (cm)		1000 Seed Weight (g)		Oil Content (%)		Protein Content (%)	
	M_3	M_4	M_3	M_4	M_3	M_4	M_3	M_4	M_3	M_4	M_3	M_4	M_3	M_4
SAMSOY (Stnd.)	$341.9^{a,b}$	297.9^{a}	15.7	10.2	46.5	$86.5^{a,c}$	89.3^{c}	$125.0^{e,f}$	$19.29^{c,d}$	$17.21^{c,d}$	$22.27^{b,c}$	$22.12^{a,d}$	$40.77^{c,f}$	$42.13^{a,d}$
SA88 (Stnd.)	NA	$288.8^{a,c}$	NA	10.0	NA	118.7^{a}	NA	$135.7^{c,f}$	NA	$16.49^{d,e}$	NA	$22.59^{a,b}$	NA	$41.39^{b,e}$
NOVA (Stnd.)	NA	214.1^{c}	NA	8.7	NA	$98.7^{a,c}$	NA	$147.8^{a,c}$	NA	19.6^{a}	NA	$21.87^{b,e}$	NA	$42.95^{a,b}$
ATAEM-7	$300.0^{a,b}$	$291.2^{a,b}$	17.8	10.0	38.8	$108.8^{a,c}$	$101.2^{b,c}$	$141.2^{a,d}$	19.98^{c}	$18.75^{a,b}$	$21.64^{b,e}$	$21.37^{c,e}$	$41.61^{a,e}$	$42.82^{a,b}$
AM1	$366.7^{a,b}$	$292.5^{a,b}$	22.7	10.2	46.5	122.2^{a}	$120.6^{a,c}$	$128.7^{d,f}$	$23.46^{a,b}$	$16.49^{d,e}$	$21.97^{b,e}$	$22.4^{a,c}$	$40.56^{c,f}$	$41.45^{b,e}$
SM1	294.4	$260.3^{a,c}$	20.2	7.3	44.7	$115.5^{a,b}$	$99.3^{b,c}$	$136.2^{c,f}$	$21.32^{b,c}$	14.22^{f}	$21.48^{b,e}$	$22.4^{a,c}$	$41.68^{a,c}$	$41.69^{b,e}$
SM2	$372.3^{a,b}$	$249.8^{a,c}$	21.6	8.3	60.8	$93.3^{a,c}$	$92.2^{b,c}$	$153.3^{a,b}$	20.5^{c}	14.77^{f}	$22.11^{b,d}$	$20.99^{d,e}$	$40.59^{c,f}$	$42.73^{a,c}$
SM2-1	293.8^{b}	$247.5^{a,c}$	19.5	10.0	49.2	$94.8^{a,c}$	$101.7^{a,c}$	122.7^{f}	$21.38^{b,c}$	$17.94^{b,c}$	21.1^{e}	20.7^{e}	42.63^{a}	43.62^{a}
SM3	$378.7^{a,b}$	$271.1^{a,c}$	16.8	11.1	50.0	67.6^{c}	$128.5^{a,b}$	156.3^{a}	24.64^{a}	$15.0^{e,f}$	22.39^{b}	$22.87^{a,b}$	$41.11^{b,f}$	39.94^{e}
SM3-1	437.6^{a}	$243.2^{a,c}$	16.4	5.3	85.2	$110.3^{a,c}$	$117.1^{a,c}$	$130.0^{c,f}$	$19.45^{c,d}$	$15.13^{e,f}$	23.49^{a}	$21.76^{b,e}$	$40.23^{e,f}$	$41.78^{a,e}$
SM4	$327.8^{a,b}$	$219.7^{b,c}$	16.2	9.7	55.0	$77.5^{b,c}$	$104.1^{a,c}$	$139.5^{a,e}$	17.46^{d}	$15.28^{e,f}$	$22.04^{b,d}$	$22.14^{a,d}$	39.84^{f}	$41.54^{b,e}$
SM5	$334.1^{a,b}$	$224.1^{a,c}$	22.0	10.8	39.7	$95.7^{a,c}$	132.5^{a}	$150.2^{a,c}$	$21.39^{b,c}$	14.76^{f}	$21.45^{c,e}$	$21.3^{c,e}$	$41.86^{a,b}$	$41.13^{c,e}$
Mean	344,7	256.7	18.9	9.0	51.6	99.6	108.7	139.3	20.9	16.32	22.0	22.05	41.1	41.74
Signific.	P<0.05	P<0.05	N.S	N.S.	P<0.05	P<0.05	P<0.05	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.05

Tab. 4: Fatty acid composition (palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid) of standard soybean varieties and mutants belonging to M_3 and M_4 field trials.

Sample	Palmitic acid		Stearic acid		Oleic acid		Linoleic Acid		Linolenic acid	
	M_3	M_4	M_3	M_4	M_3	M_4	M_3	M_4	M_3	M_4
SAMSOY (Stnd.)	NA	11.37	NA	3.96	NA	27.56	NA	49.55	NA	6.84
SA88 (Stnd.)	NA	11.43	NA	4.23	NA	22.75	NA	52.95	NA	7.7
NOVA (Stnd.)	NA	10.76	NA	3.99	NA	23.48	NA	53.92	NA	6.94
ATAEM-7	9.74	11.78	3.7	3.94	24.24	26.78	53.64	49.81	7.74	6.78
AM1	11.78	11.94	4.21	3.78	23.33	25.49	52.16	50.67	7.64	7.07
SM1	11.46	11.88	3.85	4.13	25.8	26.74	51.4	49.87	6.53	6.56
SM2	11.39	11.15	4.14	4.02	22.24	22.64	53.35	53.65	7.83	7.67
SM2-1	11.56	11.77	4.04	4.17	26.66	23.43	50.32	52.48	6.53	7.32
SM3	11.51	11.71	3.68	4.26	26.14	24.81	50.68	51.16	7.1	7.12
SM3-1	11.21	11.8	3.82	4.13	25.87	22.44	50.77	53.15	7.11	7.6
SM4	10.54	11.01	4.07	3.89	30.78	26.11	47.53	51.24	6.13	6.53
SM5	10.97	10.64	3.84	3.95	26.41	29.32	51.17	48.63	6.66	6.23

Discussion

The aim of this study is to obtain and evaluate gamma-induced salinity tolerant soybean mutants derived from S04-05 and Ataem-7 varieties. For this purpose, the salinity tolerances of the original varieties and M_4 generation mutants were evaluated through growth parameters based on average plant height and weight and biochemical parameters such as chlorophyll content, proline accumulation, lipid peroxidation and total carbohydrate content. Yield and quality analysis of the mutant plants were also performed comparing to their original varieties over parameters of average plant weight (g), first pod heights (cm), pod counts, seed yield (kg/ha), 1000 seeds weights (g), oil content (%), protein content (%), fatty acid content (%). Polymorphism between salinity tolerant mutants and their original varieties was determined by using RAPD markers.

Soybean is an important industrial crop which has limited genetic diversity due to its self-pollinating nature. There are various forward and reverse genetic approaches which are utilized directly or indirectly to improve the limited diversity. Among these, mutation breeding is a proven method which provided great contribution to improve seed quality (niacin-, tannin, anthocyanine, catechin-rich), yield, pest and pesticide resistance, abiotic stress resistance, and more (Ayan et al., 2022). Our results indicate that enhancing salinity tolerance and valuable agronomical traits as fatty acid (linolenic, oleic) content is possible in time and cost-effective manner by mutation breeding.

In mutation breeding studies, determination of optimal radiation dose for each variety to induce the highest mutation frequency with minimum adverse effects on plant survival is a prerequisite step. The type, applied dose, dose rate of irradiation must be decided accurately. Therefore, determination of GR_{30} and GR_{50} doses which reduce growth of plantlets by 30 and 50%, respectively, is a crucial step (Atak et al., 2004; Çelik et al., 2021). GR_{30} doses were calculated as 138 Gy and 265 Gy, while the GR_{50} doses were calculated as 225 Gy and 385 Gy for S04-05 and Ataem-7 varieties, respectively. Beside the growth rates, seedling survival, and fertility parameters were also dramatically decreased following the applied increasing radiation doses. Physical mutagens as gamma radiation induces mutations through genome, chromosome, or gene alterations. Therefore, detection and repair of some of these mutations delays growth and related parameters of plants (Bado et al., 2015; Oladosu et al., 2016). In the present study, the days to flowering of plants increased significantly due to these delaying effects. Nevertheless, soybean is the fifth plant in Mutant Variety Database of International Atomic Energy Agency after rice, barley, *Chrysanthemum*, and wheat. 36 of 182 soybean mutants were induced and registered by direct use of physical mutagens as Cs-137 in last decade (IAEA, 2020). Gamma-induced mutation breeding in soybean is frequently used in the development of new varieties for many characteristics such as yield, high protein content, resistance to soybean mosaic virus (SMV), early maturity, resistance to leaf rust, resistance to purple seed spot, resistance to cyst nematode (SCN), drought tolerance, super nodulation, lipoxygenase absence, temperature tolerance, low allergenicity and higher nitrogen fixation (Ayan et al. 2022). Gama-radiation applied in mutation breeding leads to high ROS levels, which can have detrimental effects on biomolecules such as water content of seeds, membrane lipids, enzymes and DNA. These harmful effects cause negativities in processes such as cell cycle, mitotic division and plant growth (Popovska et al. 2017).

150 Gy radiation dose was determined as the most efficient dose, which resulted in 13 salt resistant soybean mutants, in the present study. Salinity responses of Ataem-7 and S04-04 soybean varieties were also determined for salinity tolerance selections. Both varieties were subjected to 0, 30, 60, 90, 120 and 150 mM of NaCl. Likewise, in many publications on salinity stress research, 0 - 50 mM, 50 - 100 mM and 100 - 150 mM NaCl concentrations were applied to various plant species as low, moderate, and high salinity standards, respectively (Qados, 2011; Hariadi et al., 2015; Rahneshan et al., 2018).

In plant research, effects of various abiotic and biotic stresses are evaluated through physiological parameters such as photosynthetic pigment, TBARS and proline contents (Demiral and Türkan, 2005; Şen and Alikamanoğlu, 2013; Sekmen et al., 2014). In our evaluation, TBARS and proline contents were significantly increased in 30, 60, 90,120 and 150 mm NaCl applied original varieties, while photosynthetic pigment contents were decreased in both varieties. Based on the results, 90 mM NaCl concentration has been chosen for further salinity tolerance selections.

From a total of 12,000 irradiated soybean seeds, 13 mutant lines (5 mutants derived from Ataem-7 and 8 mutants derived from the S04-05 variety) that tolerated 90 mM NaCl treatment were identified by selection under both *in vivo* and *in vitro* conditions for three generations. This low rate is similar to other mutation breeding studies using gamma radiation (Çelik et al. 2021). Similar to other physical mutagen-induced breeding studies, DNA strand breaks and DNA rearrangements led to changes in numerous metabolic processes, including photosynthesis and carbohydrate metabolism, in the study (Arase et al. 2011). Variations occurring through gamma-induced mutation breeding can create significant changes in the metabolism of soybean plants (Meriç, 2023). The random nature and high frequency of induced mutations often lead to the death of plants. Most of the undesirable effects caused by induced mutations can be reduced by appropriate selection in each generation. In a gamma-induced

mutation breeding study in tomato, 731 viable mutants from 4000 irradiated seeds were reduced to 72 drought-tolerant mutant candidates through *in vivo* and *in vitro* selections. The rest of the mutants were reported to have off-target properties (Çelik et al., 2021).

Growth parameters such as average plant height and weight and physiological parameters such as photosynthetic pigment, TBARS, total carbohydrate and proline content were evaluated in the M_4 generation of selected salt-tolerant mutants. Salinity stress is well known to cause oxidative stress in plants and leads to oxidation of unsaturated fatty acids in cell membrane which is referred to lipid peroxidation. Lipid peroxidation has deleterious effects on membrane integrity and causes imbalance in ion homeostasis through degradation of membrane proteins. Depending on intensity and duration of the stress, it may trigger cellular death. TBARS content is a measurable by-product of lipid peroxidation, therefore, is the standard method to evaluate the level of stress (Savicka and Škute, 2010; Awasthi et al., 2017). Gamma-ray-induced salt tolerant mutants of the present study presented no evidence of oxidative damage after 7 days of salinity treatment. Low oxidative damage is mostly related to the enzymatic and non-enzymatic defense mechanisms of plants during stress. Low molecular weight compatible solutes are important components of non-enzymatic protection known as osmoprotectants. Proline, sucrose, polyols, trehalose, glycine betaine, alanine betaine are particularly interest of research (Verbruggen and Hermans, 2008; Mattioli et al., 2009; Hayat et al., 2012). In the present study, proline accumulation alterations were not statistically significant. However, mutant SM1 presented 2.27-fold increased total carbohydrate accumulation compared to the salinity stress applied control group. Carbohydrate accumulation is primarily maintained through photosynthesis. However, salinity causes ion imbalance and leads to physiological drought. Photosynthesis is one of the primary targets due to the response of plants to the physiological drought by stomatal closure. Decreased CO_2 intake and increased oxidative stress may lead to the degradation of photosynthetic pigments (Amirjani, 2011; Kong-Ngern et al., 2012). In the present study, lower oxidative stress, and the accumulation of total carbohydrate levels were also supported by photosynthetic pigment content analysis. Salt tolerant mutants present either insignificant alterations or increased profiles for photosynthetic pigment contents up to 2.07-fold compared to the treated control groups.

As an important industrial plant, quantitative and qualitative properties of the seeds have utmost importance. Parameters as average plant height and the first pod height determine the effectiveness of automated harvesting machines. The quality of soybean seeds, which are highly susceptible to mechanical damage, is determined during pre- and post-harvest periods. The type and amount of mechanical damage may change viability and vigor of the seeds (Shelar, 2008). In this regard, average plant height values presented significant increases even though the first pod height changes were insignificant. All the mutants were found in suitable range of automated harvesting with minimum mechanical damage. As most of the agricultural plants, improving seed yield and 1000-seed weights are the most important objective in soybean breeding. Seed yield is a complex trait which should also be evaluated in association with several other factors such as seed size, days to flowering, days to maturity, seed filling period and pod count per plant. Inheritance properties and environmental factors have combined effects on these traits (Dargahi et al., 2014). In the present study, both yield and 1000-seed weights presented significant increases among induced mutants. Particularly, SM3 presented the highest yield by 437.6 kg/da. Average pod counts also showed statistically significant increases per plant.

Along with these quantitative parameters, qualitative properties as oil content, fatty acid composition and protein content are beneficial for industrial and bioenergy uses (Sultan et al., 2015). According to the USDA, soybean oil takes the second place in vegetable oil production following palm oil. In contrast to other beans, soybean has notable polyunsaturated fatty acid contents as linoleic (≅ 55%) and linolenic acids (≅ 10%), considerable amount of monounsaturated fatty acids as oleic acid (≅ 18%), and saturated fatty acids as palmitic (≅ 13%) and stearic acids (≅ 4%) in lesser levels. Linoleic acid is the most abundant fatty acid which constitutes approximately half of the total fatty acid content. Soybean is also a major provider of an essential fatty acid component of human diet called α-linolenic acid (Graef et al., 2009; Prabakaran et al., 2018). In this mutation breeding study, protein and oil contents of selected salt tolerant mutants presented significant increases. The most abundant fatty acids were linoleic and oleic acids, respectively. As a nutritional α-linolenic acid value, SM2 mutant presented the highest level among the other mutants.

Conclusion

90 mM NaCl tolerant soybean mutants were induced by gamma radiation. 13 different mutants were selected under *in vivo* conditions and the permanence of the tolerance was confirmed in *in vitro* conditions, by screening three generations. Mutants presented significant fatty acid alterations. Average oleic acid contents were increased, compared to the accepted soybean standard fatty acid values, in an amount of the decrease in linolenic acid content. It has also been emphasized with this study that mutation breeding is cost and time effective and safe method for obtaining elite varieties in terms of multiple quality characteristics from an agricultural point of view.

Acknowledgements

This study was financially supported by the Republic of Turkey Ministry of Agriculture and Forestry General Directorate of Agricultural Research and Policies (TAGEM) (Grant Number: TAGEM-17-AR-GE-56).

Conflict of interest

No potential conflict of interest was reported by the authors.

References

Amirjani, M.R., 2011: Effect of salinity stress on growth, sugar content, pigments and enzyme activity of rice. Int. J. Bot. 7(1), 73-81. DOI: 10.3923/ijb.2011.73.81

Arase, S., Hase, Y., Abe, J., 2011: Optimization of ion-beam irradiation for mutagenesis in soybean: effects on plant growth and production of visibly altered mutants. Plant Biotech. 28(3), 323-329. DOI: 10.5511/plantbiotechnology.11.0111a

Arnon, D.I., 1949: Copper enzymes in isolated chloroplasts: polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24(1), 1-15. DOI: 10.1104/pp.24.1.1

Atak, C., Alikamanoğlu, S., Açık, L., Canbolat, Y., 2004: Induced of Plastid Mutations in Soybean Plant (*Glycine max* L. Merrill) with gamma radiation and determination with RAPD. Mutat. Res. Mol. Mech. Mutagen. 556(1), 35-44. DOI: 10.1016/j.mrfmmm.2004.06.037

Awasthi, J.P., Saha, B., Regon, P., Sahoo, S., Chowra, U., Pradhan, A., Roy, A., Panda, S.K., 2017: Morpho-physiological analysis of tolerance to aluminum toxicity in rice varieties of North East India. PLoS One. 12(4), e0176357. DOI: 10.1371/journal.pone.0176357

Ayan, A., Meriç, S., Gümüş, T., Atak, Ç., 2022: Current strategies and future of mutation breeding in soybean improvement. Soybean - Recent Adv. Res. Appl. InTecOpen. DOI: 10.5772/intechopen.104796

Bado, S., Forster, B.P., Nielsen, S., Ali, A.M., Lagoda, P.J.L., Till, B.J., Laimer, M., 2015: Plant mutation breeding: current progress and future assessment. In: Plant Breed. Rev. Vol. 39, 23-88. DOI: 10.1002/9781119107743.ch2

Bates, L.S., Waldren, R.P., Teare, I.D., 1973: Rapid determination of free proline for water-stress studies. Plant Soil. 39(1), 205-207. DOI: 10.1007/BF00018060

Çelik, Ö., Ayan, A., Meriç, S., Atak, Ç., 2021: Comparison of tolerance related proteomic profiles of two drought tolerant tomato mutants improved by gamma radiation. J. Biotechnol. 330, 35-44. DOI: 10.1016/j.jbiotec.2021.02.012

Dargahi, H., Tanya, P., Somta, P., Abe, J., Srinives, P., 2014: Mapping quantitative trait loci for yield-related traits in soybean (*Glycine max* L.). Breed Sci. 64(4), 282. DOI: 10.1270/jsbbs.64.282

Demiral, T., Türkan, I., 2005: comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Environ. Exp. Bot. 53(3), 247-257. DOI: 10.1016/j.envexpbot.2004.03.017

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956: Colorimetric method for determination of sugars and related substances. Anal. Chem. 28(3), 350-356. DOI: 10.1021/ac60111a017

FAO, 2022: FAOSTAT. Retrieved 27th March 2022, from https://www.fao.org/faostat/en/#data/QCL

Graef, G., Lavallee, B.J., Tenopir, P., Tat, M., Schweiger, B., Kinney, A.J., Van Gerpen, J.H., Clemente, T.E., 2009: A high-oleic-acid and low-palmitic-acid soybean: agronomic performance and evaluation as a feedstock for biodiesel. Plant Biotechnol. J. 7(5), 411-421. DOI: 10.1111/j.1467-7652.2009.00408.x

Hariadi, Y.C., Nurhayati, A.Y., Soeparjono, S., Arif, I., 2015: Screening six varieties of rice (*Oryza sativa*) for salinity tolerance. Procedia Environ. Sci. 28, 78-87. DOI: 10.1016/j.proenv.2015.07.012

Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J., Ahmad, A., 2012: Role of proline under changing environments: a review. Plant Signal Behav. 7(11), 1456-1466. DOI: 10.4161/psb.21949

Hoagland, D.R., Arnon, D.I., 1950: The water-culture method for growing plants without soil. Circ. Calif. Agric. Exp. Stn. 347(2nd edit), 32.

IAEA, 2020: Mutant Variety Database. Retrieved 28th August 2020, from https://mvd.iaea.org/.

IAEA, 2022: Mutant Variety Database (MVD). Retrieved 7th February 2022, from https://mvd.iaea.org/#!Home.

Jegadeesan, S., Punniyamoorthy, D., 2023: Potential of Mutation Breeding in Genetic Improvement of Pulse Crops. In: Penna, S., Jain, S.M. (eds.), Mutation Breeding for Sustainable Food Production and Climate Resilience. Springer, Singapore. DOI: 10.1007/978-981-16-9720-3_15

Kong-Ngern, K., Bunnag, S., Theerakulpisut, P., 2012: Proline, hydrogen peroxide, membrane stability and antioxidant enzyme activity as potential indicators for salt tolerance in rice (*Oryza sativa* L.). Int. J. Bot. 8(2), 54-65. DOI: 10.3923/ijb.2012.54.65

Mattioli, R., Costantino, P., Trovato, M., 2009: Proline accumulation in plants: not only stress. Plant Signal Behav. 4(11), 1016-1018. DOI: 10.4161/psb.4.11.9797

Meriç, S., Ayan, A., Atak, Ç., Ari, Ş., 2023: Profile-based proteomic investigation of unintended effects on transgenic and gamma radiation induced mutant soybean plants. Genet Resour. Crop Evol. 70, 2077-2095. DOI: 10.1007/s10722-023-01560-5

Oladosu, Y., Rafii, M.Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H.A., Miah, G., Usman, M., 2016: Principle and application of plant mutagenesis in crop improvement: a review. Biotechnol. Equip. 30(1), 1-16. DOI: 10.1080/13102818.2015.1087333

Popovska, M., Popovski, B., Gjamovski, V., 2017: Primary effects of gamma radiation (Cz137) on the morphology of leaves at some sweet cherry varieties. JAFES 71(2), 148-154.

Prabakaran, M., Lee, K.J., An, Y., Kwon, C., Kim S., Yang, Y., Ahmad, A., Kim, S.H., Chung, I.M., 2018: Changes in soybean (*Glycine max* L.) flour fatty-acid content based on storage temperature and duration. Molecules 23(10), 2713. DOI: 10.3390/molecules23102713

Qados, A.M.S.A., 2011: Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.). J. Saudi Soc. Agric. Sci. 10(1), 7-15. DOI: 10.1016/j.jssas.2010.06.002

Rahneshan, Z., Nasibi, F., Moghadam, A.A., 2018: Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera* L.) rootstocks. J. Plant Interact. 13(1), 73-82. DOI: 10.1080/17429145.2018.1424355

Rao, K.V.M., Sresty, T.V.S., 2000: Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. Plant Sci. 157(1), 113-128. DOI: 10.1016/S0168-9452(00)00273-9

Reddy, I.N.B.L., Kim, B.-K., Yoon, I.-S., Kim, K.-H., Kwon, T.-R., 2017: Salt Tolerance in Rice: Focus on Mechanisms and Approaches. Rice Sci. 24(3), 123-144. DOI: 10.1016/j.rsci.2016.09.004

Savicka, M., Škute, N., 2010: Effects of high temperature on malondialdehyde content, superoxide production and growth changes in wheat seedlings (*Triticum aestivum* L.). Ekologija. 56(1), 26-33. DOI: 10.2478/v10055-010-0004-x

Sekmen, A.H., Özgür, R., Uzildaz B., Türkan, I., 2014: Reactive oxygen species scavenging capacities of cotton (*Gossypium hirsutum*) cultivars under combined drought and heat induced oxidative stress. Environ. Exp. Bot. 99, 141-149. DOI: 10.1016/j.envexpbot.2013.11.010

Şen, A., Alikamanoğlu, S., 2013: Antioxidant enzyme activities, malondialdehyde, and total phenolic content of peg-induced hyperhydric leaves in sugar beet tissue culture. Vitr. Cell Dev. Biol. 49(4), 396-404. DOI: 10.1007/s11627-013-9511-2

Shelar, V.R., 2008: Role of mechanical damage in deterioration of soybean seed quality during storage - a review. Agric. Rev. 29(3), 177-184.

Stewart, R.R.C., Bewley, J.D., 1980: Lipid-peroxidation associated with accelerated aging of soybean axes. Plant Physiol. 65(2), 245-248. DOI: 10.1104/pp.65.2.245

Sultan, S.M., Dikshit, N., Vaidya, U.J., 2015: Oil content and fatty acid composition of soybean (*Glycine max* l.) genotypes evaluated under rainfed conditions of Kashmir himalayas in India. J. Appl. Nat. Sci. 7(2), 910-915. DOI: 10.31018/jans.v7i2.706

Verbruggen, N., Hermans, C., 2008: Proline accumulation in plants: a review. Amino Acids. 35(4), 753-759. DOI: 10.1007/s00726-008-0061-6

Wagner, S.C., 2011: Biological nitrogen fixation. Nat. Educ. Knowl. 3(10), 15.

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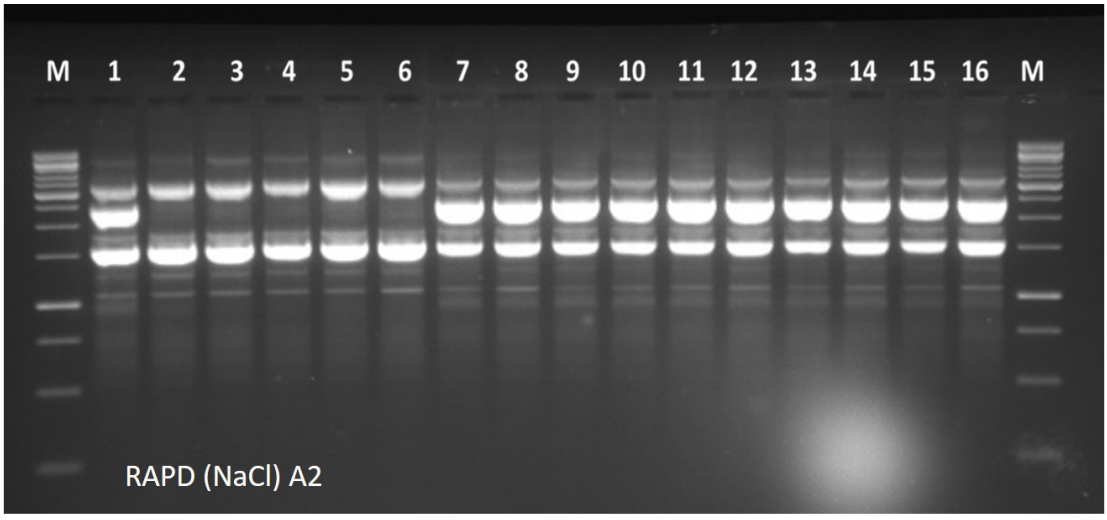
Supplementary material



1: Ataem-7 Control (+), 2: Ataem-7 Control(-), 3: AM1(+), 4: AM1(-), 5: S04-05 Control(+), 6: S04-05 Control(-), 7: SM2(+), 8: SM2(-), 9: SM3(+), 10: SM3(-), 11: SM4(+), 12: SM4(-), 13: SM5(+), 14: SM5(-), 15: SM1(+), 16: SM1(-), M: Marker (Thermo GeneRuler 1 kb DNA Ladder)

Fig. S1: Representative polymorphic band patterns obtained by RAPD primer of A2

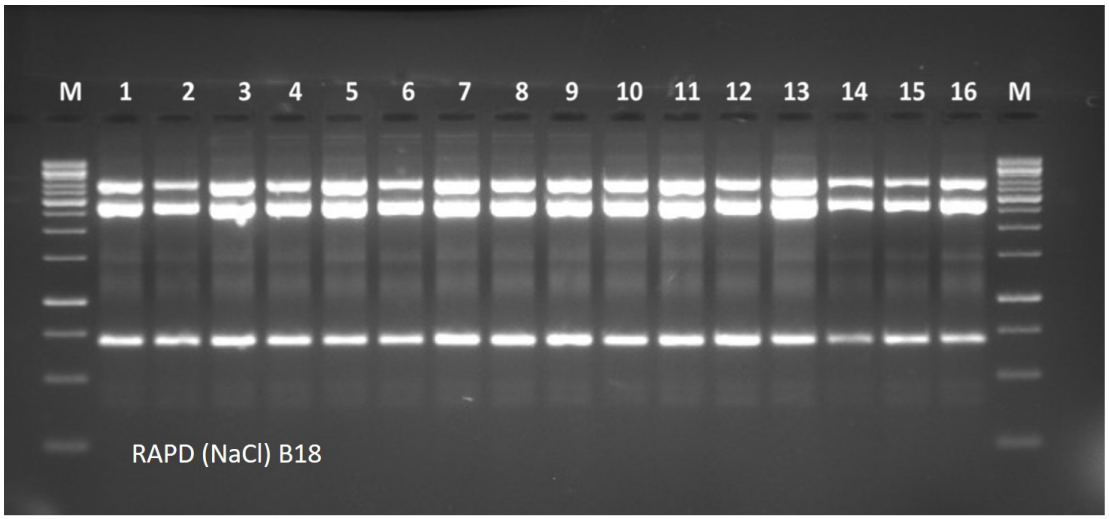


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Fig. S2: Representative polymorphic band patterns obtained by RAPD primer of B18

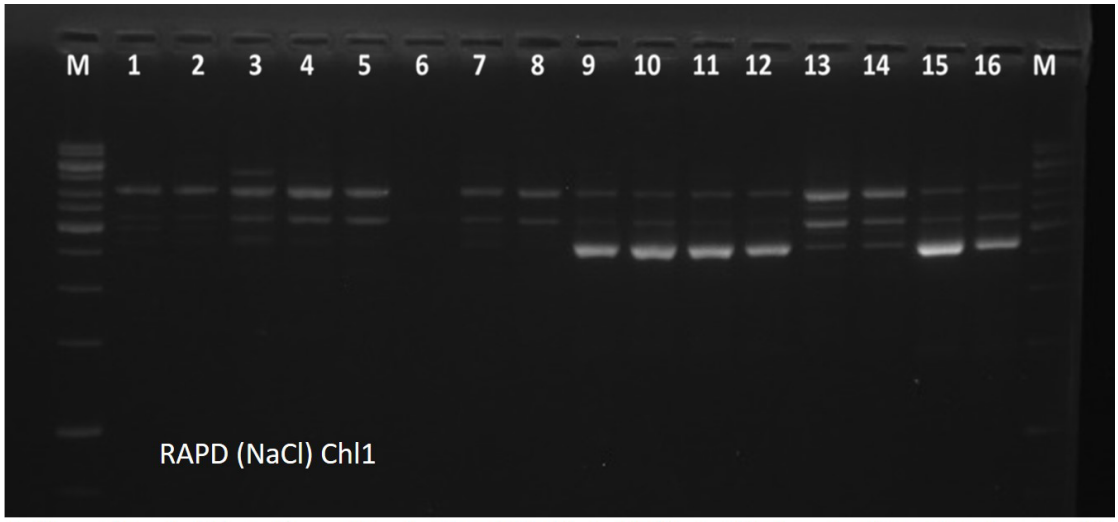

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Fig. S3: Representative polymorphic band patterns obtained by RAPD primer of Chl1

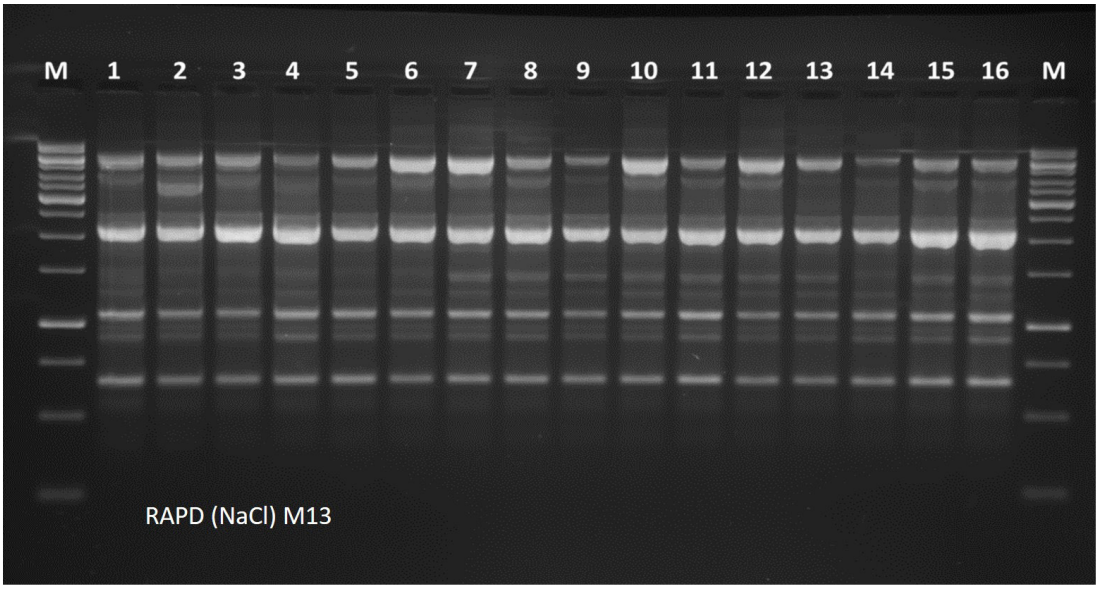

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Fig. S4: Representative polymorphic band patterns obtained by RAPD primer of M13

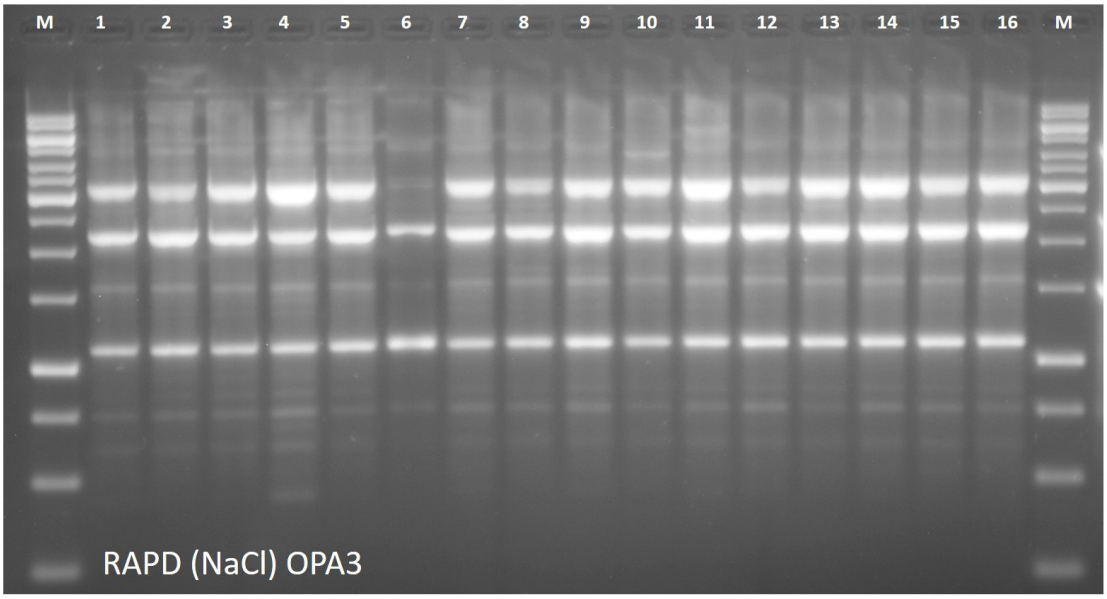

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Fig. S5: Representative polymorphic band patterns obtained by RAPD primer of OPA3

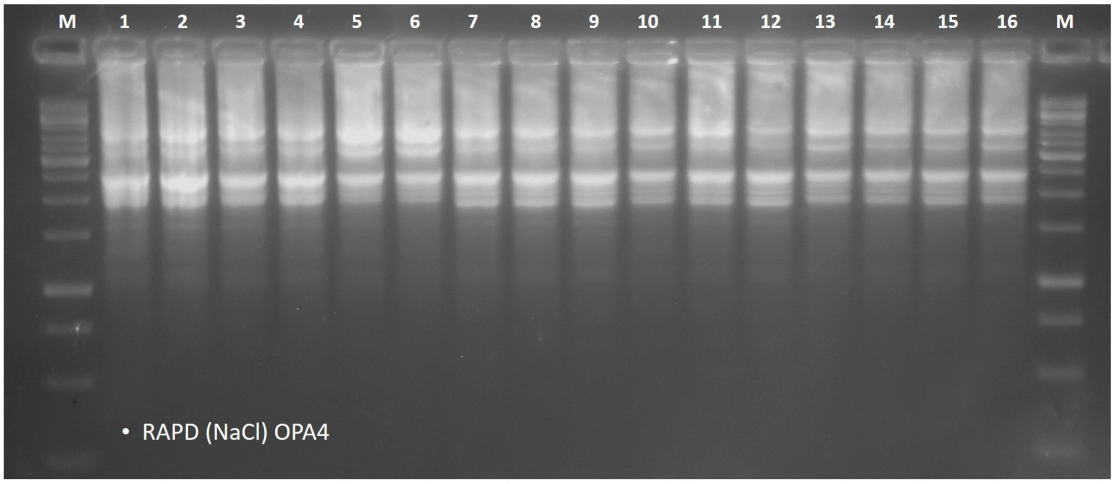

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Fig. S6: Representative polymorphic band patterns obtained by RAPD primer of OPA4

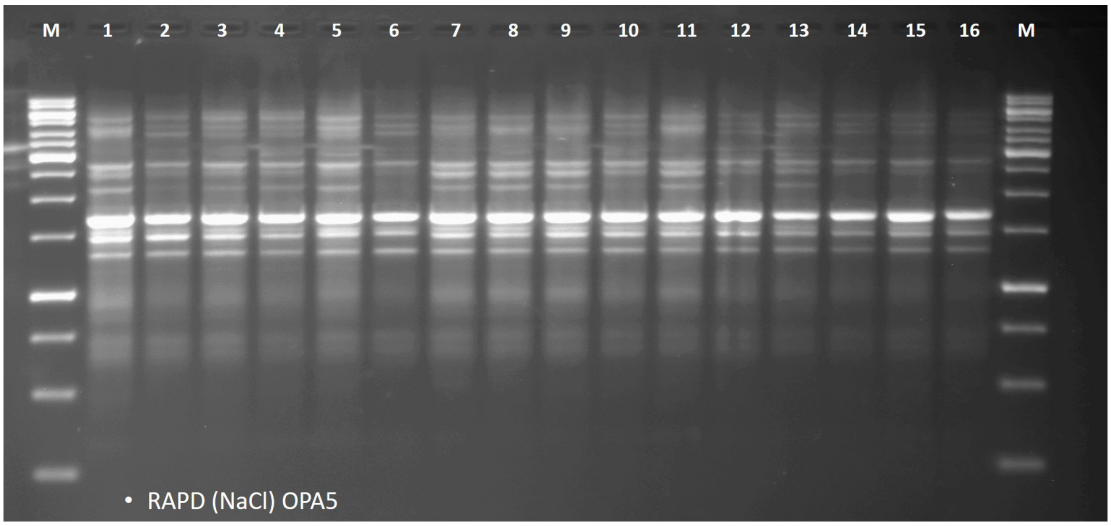

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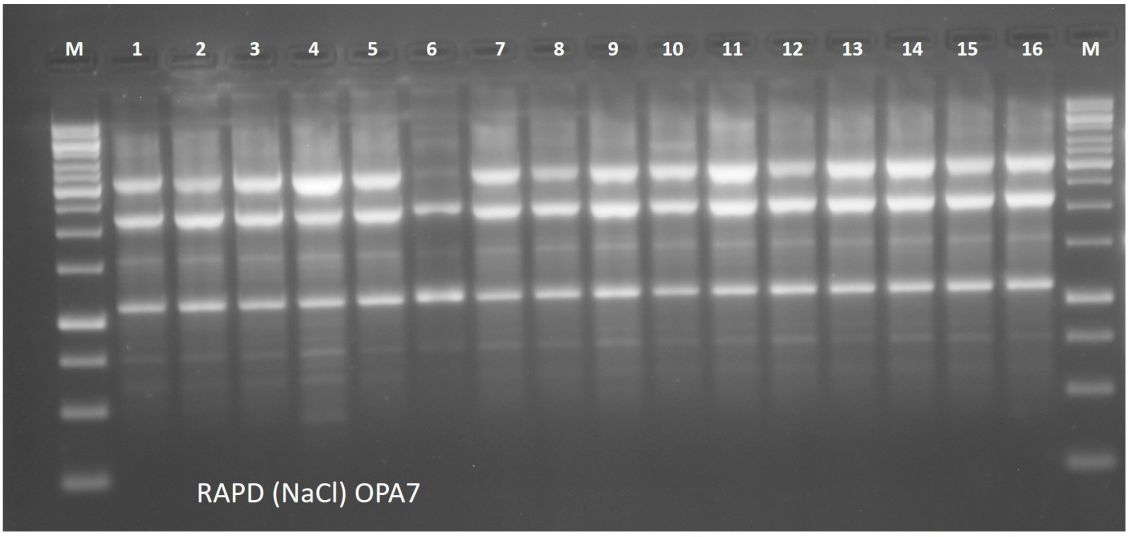
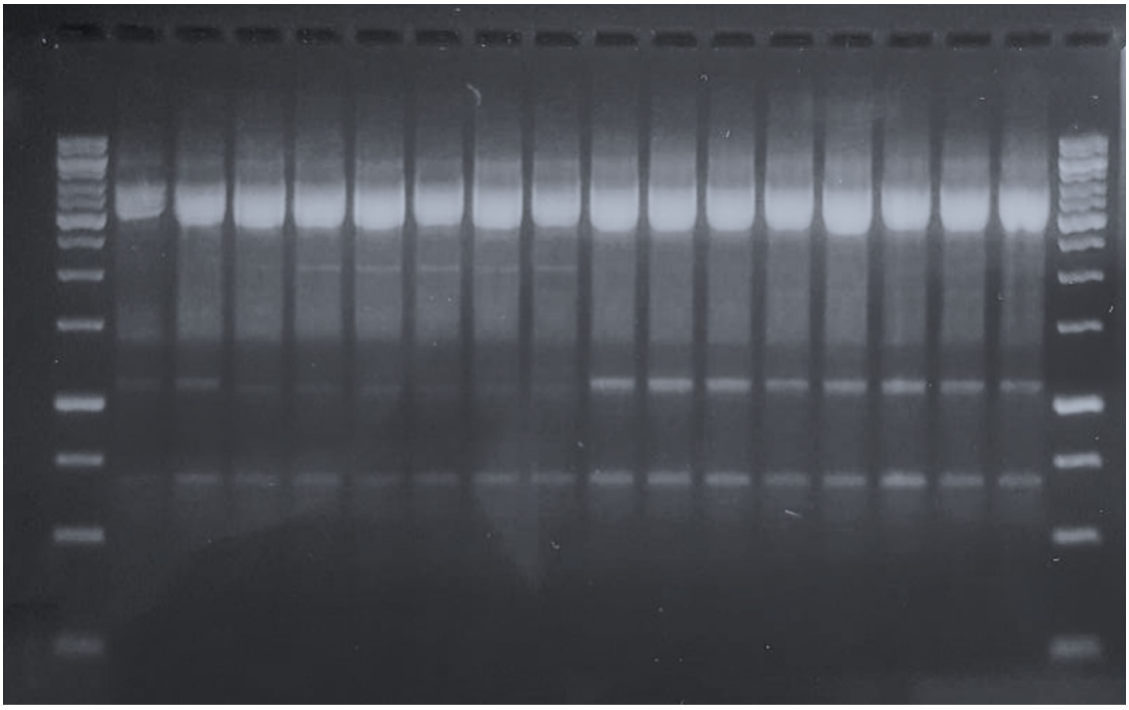
Fig. S7: Representative polymorphic band patterns obtained by RAPD primer of OPA5



1: Ataem-7 Control (+), 2: Ataem-7 Control(-), 3: AM1(+), 4: AM1(-), 5: S04-05 Control(+), 6: S04-05 Control(-), 7: SM2(+), 8: SM2(-), 9: SM3(+), 10: SM3(-), 11: SM4(+), 12: SM4(-), 13: SM5(+), 14: SM5(-), 15: SM1(+), 16: SM1(-), M: Marker (Thermo GeneRuler 1 kb DNA Ladder)

Fig. S8: Representative polymorphic band patterns obtained by RAPD primer of OPA7



1: Ataem-7 Control (+), 2: Ataem-7 Control(-), 3: AM1(+), 4: AM1(-), 5: S04-05 Control(+), 6: S04-05 Control(-), 7: SM2(+), 8: SM2(-), 9: SM3(+), 10: SM3(-), 11: SM4(+), 12: SM4(-), 13: SM5(+), 14: SM5(-), 15: SM1(+), 16: SM1(-), M: Marker (Thermo GeneRuler 1 kb DNA Ladder)

Fig. S9: Representative polymorphic band patterns obtained by RAPD primer of OPA8

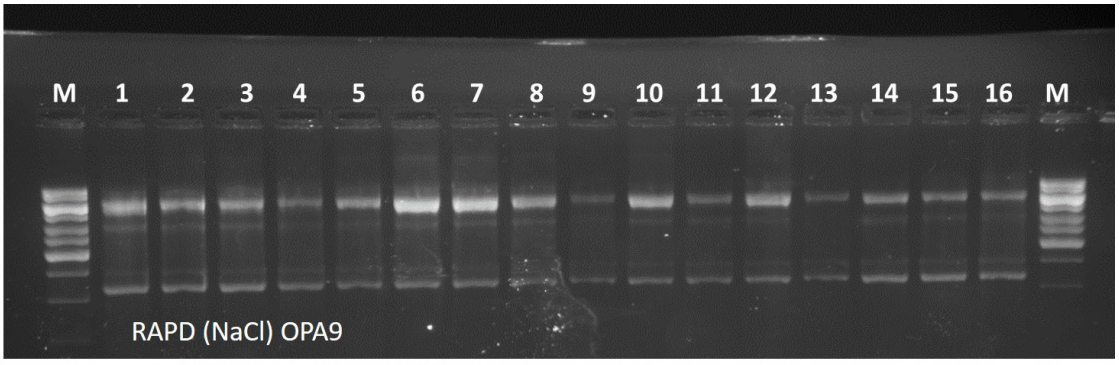


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Fig. S10: Representative polymorphic band patterns obtained by RAPD primer of OPA9

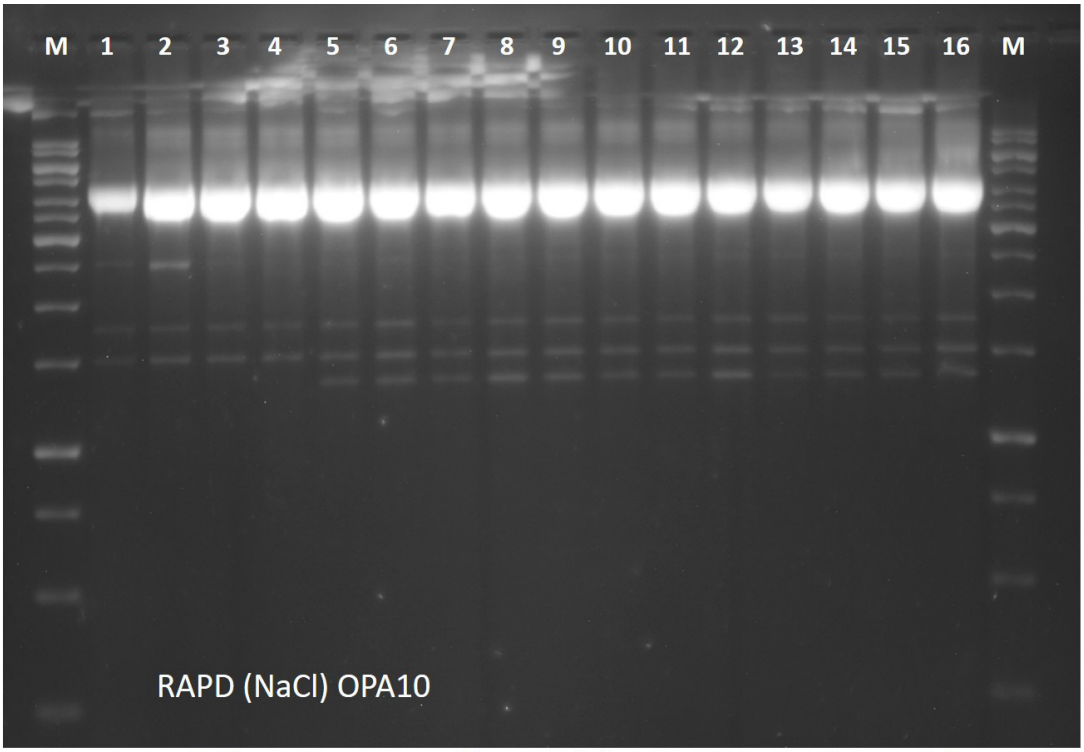

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Fig. S11: Representative polymorphic band patterns obtained by RAPD primer of OPA10

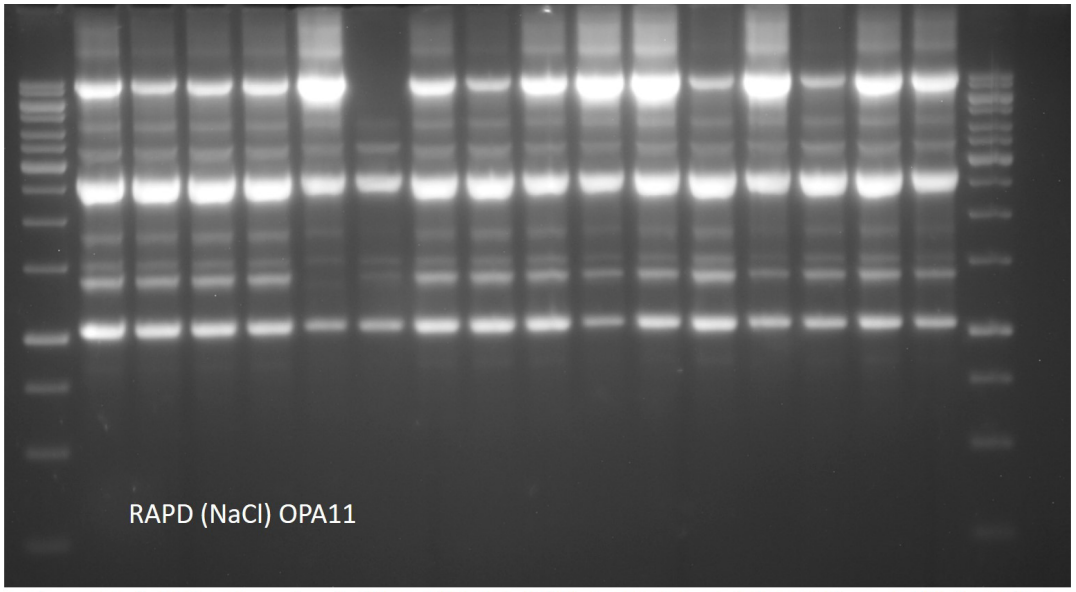

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Fig. S12: Representative polymorphic band patterns obtained by RAPD primer of OPA11

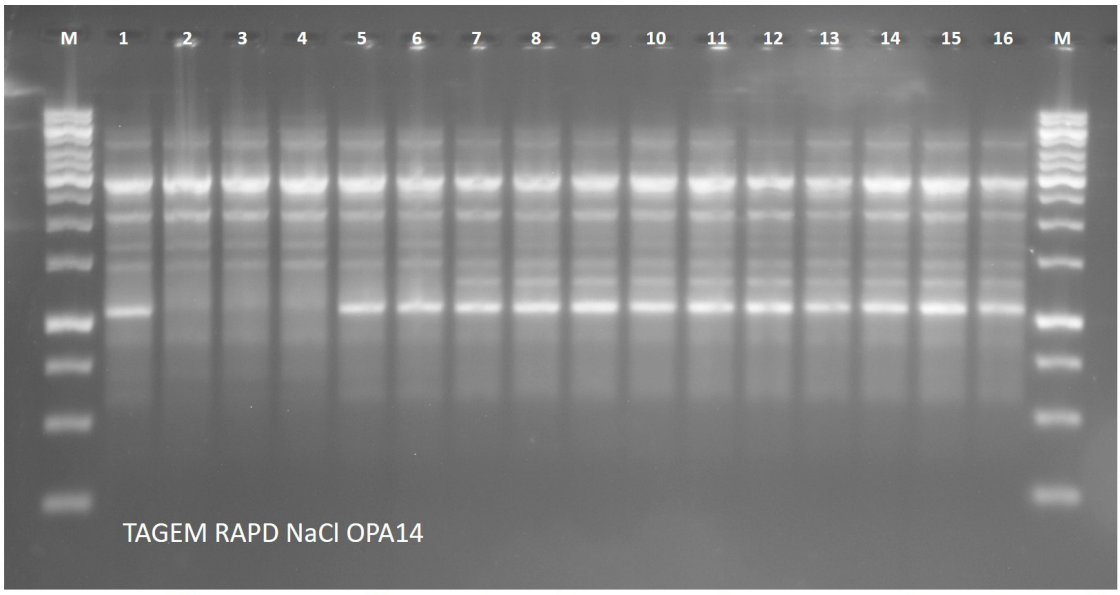

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Fig. S13: Representative polymorphic band patterns obtained by RAPD primer of OPA14

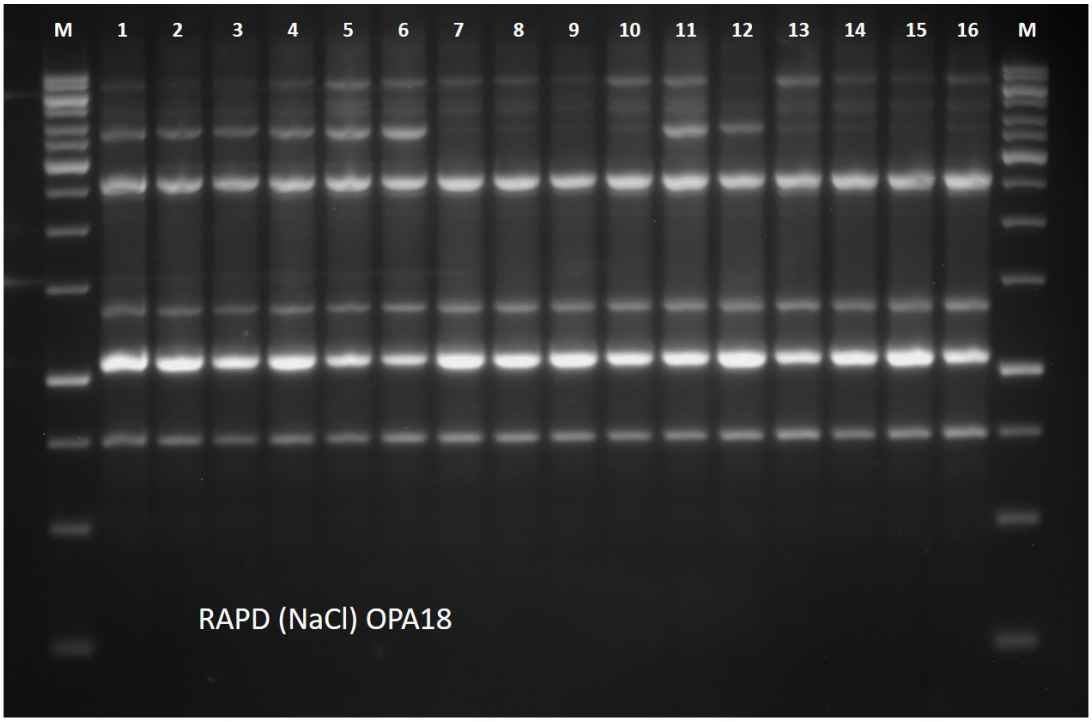

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Fig. S14: Representative polymorphic band patterns obtained by RAPD primer of OPA18

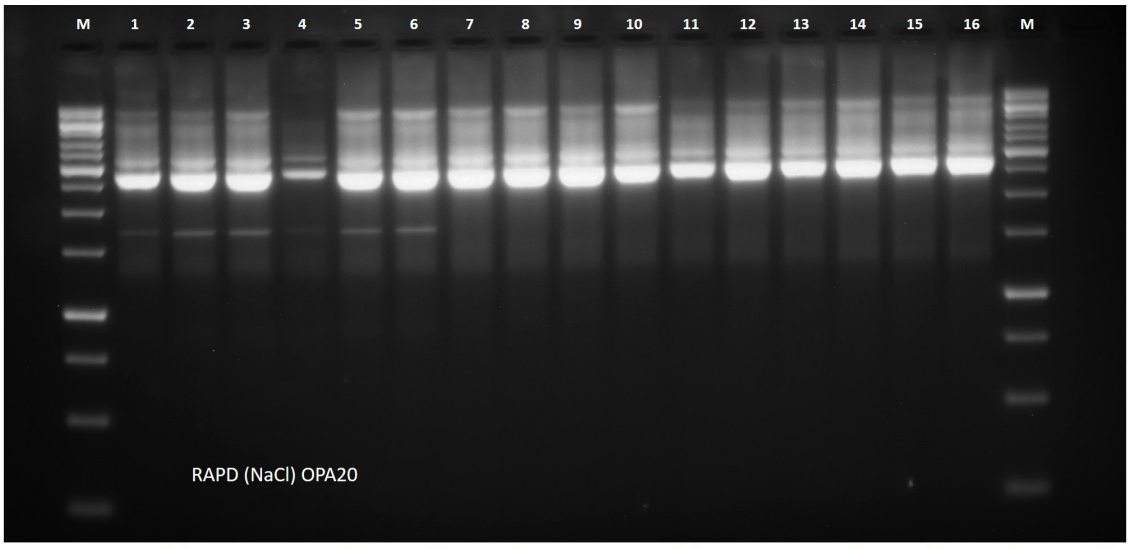

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Fig. S15: Representative polymorphic band patterns obtained by RAPD primer of OPA20

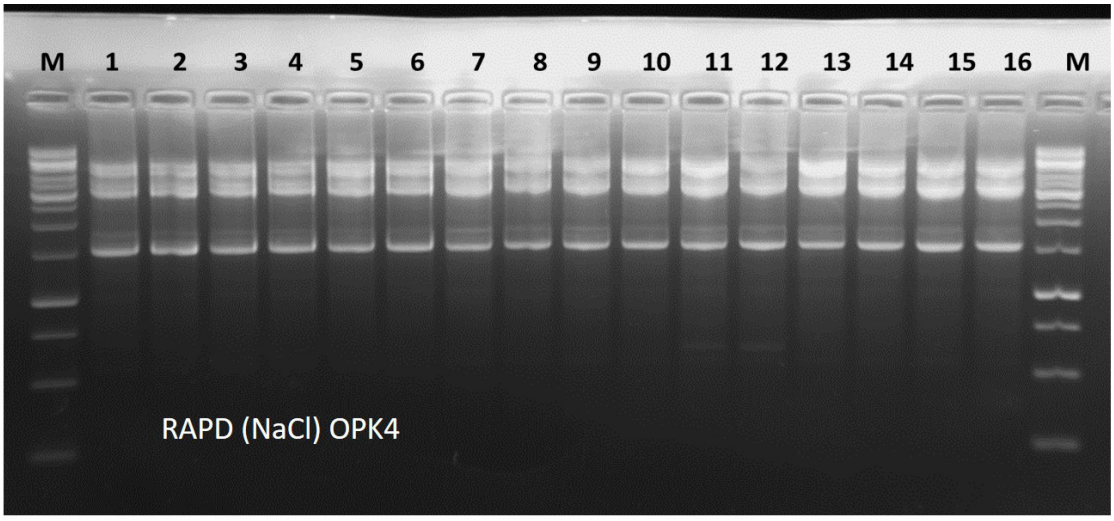

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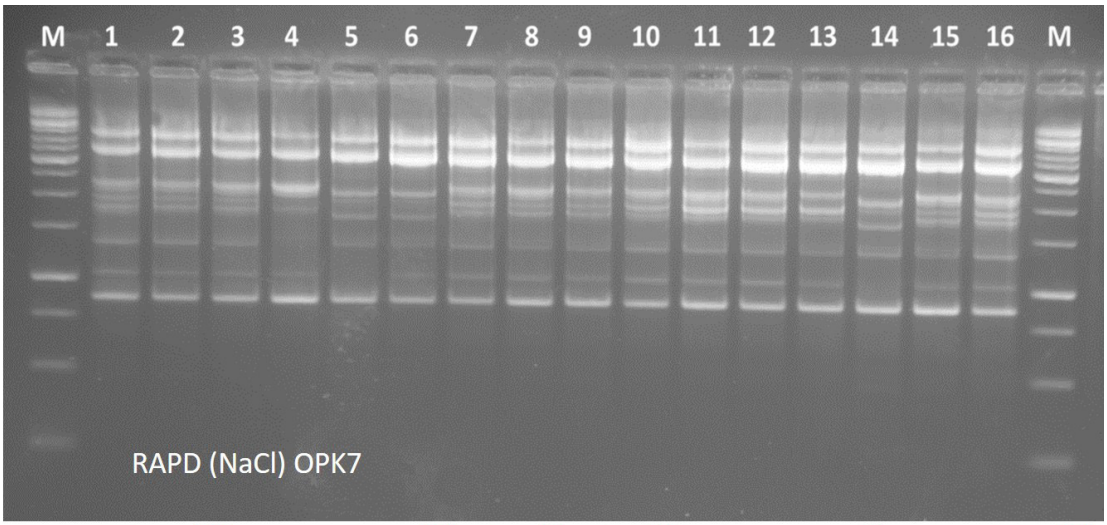
Fig. S16: Representative polymorphic band patterns obtained by RAPD primer of OPK4



1: Ataem-7 Control (+), 2: Ataem-7 Control(-), 3: AM1(+), 4: AM1(-), 5: S04-05 Control(+), 6: S04-05 Control(-), 7: SM2(+), 8: SM2(-), 9: SM3(+), 10: SM3(-), 11: SM4(+), 12: SM4(-), 13: SM5(+), 14: SM5(-), 15: SM1(+), 16: SM1(-), M: Marker (Thermo GeneRuler 1 kb DNA Ladder)

Fig. S17: Representative polymorphic band patterns obtained by RAPD primer of OPK7