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# Morpho-Molecular Screening of Rice Landraces (*Oryza sativa* L.) for Combined Salinity and Submergence Tolerance

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**ABSTRACT**

This study aimed to identify rice landraces exhibiting dual tolerance to salinity and submergence using a hydroponic screening system. Thirty-day-old seedlings of 19 rice landraces and six check varieties were exposed to salinity stress (8 and 12 dS/m, imposed using crude sea salt) and complete submergence under both freshwater and saline (8 dS/m) conditions. Morphological screening, based on a composite stress tolerance score integrating germination performance, growth response, and biomass reduction, identified Kakua as the most tolerant genotype. Several landraces, including Sona joly, Nazira shail, Sylhet balam, and Bhagnoli, exhibited moderate tolerance. Genetic diversity analysis using SSR markers (RM585 and SC3) revealed distinct clusters. Landraces such as Goccha, Machranga, and Biran grouped with the tolerant check variety Binadhan 10, while Sylhet balam, Dud shail, and Kakua showed a close genetic association with Binadhan 11. By integrating morphological and molecular data, the study identifies Kakua, Sylhet balam, Dud shail, Nonia, Roa, Sona joly, Goccha, and Machranga as promising genetic resources for breeding programs aimed at improving dual tolerance to salinity and submergence in rice.

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## Introduction

The coastal region of Bangladesh comprises 19 districts and covers 47,201 km², accounting for 32% of the country's total area. With a population of approximately 35 million, this zone supports 29% of the nation's inhabitants (Islam et al., 2009; MoWR, 2005). Rice paddies in this region are subjected to the dual and often sequential stresses of salinity and submergence within a single growing season, a result of seasonal flooding and seawater intrusion. Salinity stress presents two major challenges for rice plants: osmotic stress and ionic stress. Osmotic stress immediately reduces water uptake, visibly stunting shoot growth. Ionic stress manifests later, as toxic sodium ions ($Na^+$) accumulate in leaves, leading to premature senescence of older tissues and ultimately disrupting vital processes like photosynthesis, enzyme activity, and protein synthesis (Horie et al. 2012). Compounding this saline environment, flash floods cause sudden submergence stress. These rapid surges of water, which typically recede within 10 days, are a common phenomenon in parts of the coastal zone, further challenging rice survival.

Bangladesh is endowed with a rich diversity of traditional rice landraces. Although often low-yielding under optimal conditions, these landraces represent invaluable genetic reservoirs, frequently harboring genes for high yield stability and resilience to biotic and abiotic stresses. Systematic characterization and utilization of these landraces are therefore essential for identifying donor parents with traits such as salinity and submergence tolerance, which can be introduced into modern high-yielding varieties to ensure sustainable production (Emon and Ahammed, 2020).

Phenotype based genetic diversity is not authentic enough due to the interaction between gene and environment. To address these challenges efficiently, modern breeding techniques offer powerful tools. Marker-Assisted Selection (MAS) provides an economic, efficient, and less time-consuming alternative to conventional breeding (Schulman, 2007; Sangeetha et al., 2020). Specifically, Simple Sequence Repeat (SSR) markers are highly effective for genetic analysis, enabling DNA fingerprinting, assessment of genetic relationships, and the identification of quantitative trait loci (QTLs) associated with stress tolerance (Hoshino et al., 2012). This approach allows for the precise integration of desirable genetic material from landraces into elite breeding lines, accelerating the development of resilient cultivars.

In light of these challenges, the present study was formulated to identify climate-resilient rice varieties through an integrated morpho-molecular approach, with the specific objectives of (i) screening a diverse collection of rice germplasm for tolerance to combined salinity and submergence stresses at the critical seedling stage, and (ii) employing SSR marker based DNA fingerprinting to elucidate the genetic relationships and diversity among 19 selected rice landraces, thereby identifying potential donors for future breeding programs.

## Materials and Methods

### Phenotyping

#### Collection of plant materials, experimental design, and data collection

Seeds of different rice varieties (Table 1) were collected from Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh and Bangladesh Rice Research Institute (BRRI), Gazipur. The collected seeds were stored in refrigerator till use for experimental purpose.

**Table 1.** List of rice genotypes used for the study

| Accession no. | Name of germplasms | Types |
|---|---|---|
| G1 | Agar | Landrace |
| G2 | Gajor goria | Landrace |
| G3 | Bina shail | Landrace |
| G4 | Bogi | Landrace |
| G5 | Kakua | Landrace |
| G6 | FL-378 | Moderately salinity Tolerant |
| G7 | Sona joly | Landrace |
| G8 | Binadhan 7 | Submergence + salinity susceptible |
| G9 | Nazira shail | Landrace |
| G10 | Sylhet balam | Landrace |
| G11 | Baghnoli | Landrace |
| G12 | Ashi binni | Landrace |
| G13 | Binadhan 10 | Salinity Tolerant |
| G14 | Talmuri | Landrace |
| G15 | Dud shail | Landrace |
| G16 | Anik | Landrace |
| G17 | FL-478 | Salinity Tolerant |
| G18 | Boiri | Landrace |
| G19 | Roa | Landrace |
| G20 | Machranga | Landrace |
| G21 | Nonia | Landrace |
| G22 | Binadhan 8 | Salinity Tolerant |
| G23 | Goccha | Landrace |
| G24 | Biran | Landrace |
| G25 | Binadhan 11 | Submergence Tolerant |

To investigate phenotypic changes under stress, a germination trial was initiated using 100 rice seeds per genotype under salt stress. Germination was assessed at two salinity levels such as 0 dS/m (control) and 8 dS/m, established by dissolving crude salt in distilled water. Germination percentage was recorded after 1 day of seed placement and continues up to 5 days of seed placement. The data (such as germination rate, percent reduction in agronomical parameters) obtained and derived from the experiment.

Following germination, uniformly germinated seeds were transferred to a hydroponic system using plastic trays (35 cm × 30 cm × 4 cm), following an established IRRI protocol (Gregorio et al., 1997). The experiment comprised five distinct treatments: a non-salinized control, two salinity levels (8 dS/m and 12 dS/m), a submergence-only treatment (in salt-free water), and a combined salinity-plus-submergence treatment (at 8 dS/m). All treatments were maintained with two replications.

The hydroponic nutrient solution was prepared using Peters Professional® (20-20-20) water-soluble fertilizer, supplying 20% N, 20% $P_2O_5$, and 20% $K_2O$. A stock solution was created by dissolving 1 g of fertilizer per liter of tap water, supplemented with 110 mg/L of Ferrous Sulphate ($FeSO_4 \cdot 7H_2O$) to prevent iron deficiency. Target salinity levels were achieved by amending the nutrient solution with crude salt. The submergence environment was simulated by completely immersing the seedlings.

Stress tolerance was evaluated based on visual symptoms of stress. A modified Standard Evaluation System (SES) (Gregorio et al., 1997) was employed to score the seedlings at 12 and 30 days after the imposition of salinity and submergence stress, facilitating the classification of genotypes as tolerant, moderately tolerant, or susceptible.

Phenotypic data were collected from plants under control, salinity, submergence, and combined salinity-submergence conditions following the IRRI Standard Evaluation System (SES) (Gregorio et al., 1997). After screening, leaves, shoots, and roots from individual plants were harvested in the glasshouse. Samples were placed in separate envelopes and dried in a convection oven at 60°C for five days until a constant weight was achieved. Dry weights were measured using a digital balance.

The percent reduction in growth parameters for each stress treatment was calculated relative to the non-stressed control plants using the following formula:

Percent Reduction (%) = [ (Control Value - Stress Value) / Control Value] × 100

This calculation was applied to determine the following variables:

% Reduction in Total Dry Weight (TDW): TDW was calculated as the sum of the dry weights of leaves, shoots, and roots.

% Reduction in Total Plant Height (TPH): TPH was calculated as the sum of the shoots and roots length.

% Reduction in Total Fresh Weight (TFW): TFW was calculated as the sum of the fresh weights of leaves, shoots, and roots.

**Genotyping**

Genomic DNA was isolated from young leaves collected from 21-day-old seedlings using the IRRI miniprep protocol. To assess genetic diversity, two SSR primer pairs were selected based on their positions on the published rice microsatellite framework map. These primers were used to genotype the panel of 25 rice varieties (Table 2).

**Table 2.** The sequence and size of the microsatellite markers (SSRs) used for primer selection

| Primer name | Chromosome position (Mb) | Primer sequence | | Annealing temp. (°C) | Expected PCR product size (bp) |
|---|---|---|---|---|---|
| SC3 | 6.8 | Fwd | GCTAGTGCAGGGTTGACACA | 55 | 217 |
| | | Rev. | CTCTGGCCGTTTCATGGTAT | | |
| RM585 | 3.20 | Fwd | CAGTCTTGCTCCGTTTGTTG | 55 | 233 |
| | | Rev | CTGTGACTGACTTGGTCATAGG | | |

*Motif of the SSR maskers and number of repeats are previously published (http://www.gramene.org).

**SSR Marker Analysis**

Both SSR markers exhibited clear polymorphism across the 25 rice genotypes, confirming their utility for genetic diversity analysis. Details regarding the original source, repeat motifs, primer sequences, expected product size, chromosomal location, and repeat type for each marker are available in the Gramene database (http://www.gramene.org). PCR amplification was performed in a 10.0 µl reaction volume containing: 3.0 µl template DNA (20 ng/µl), 1.0 µl of 10X reaction buffer, 1.2 µl $MgCl_2$ (25 mM), 0.2 µl dNTPs (10 mM), 0.5 µl of each forward and reverse primer (10 µM), 0.2 µl *Taq* DNA polymerase (5 U/µl), and 3.4 µl sterile distilled water. The thermocycling profile consisted of an initial denaturation at 94°C for 5 minutes; followed by 34 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 2 minutes; with a final extension at 72°C for 7 minutes.

The PCR products were resolved by electrophoresis on 8% polyacrylamide gels in 1X TBE buffer using a vertical electrophoresis system. Gels were stained with ethidium bromide (10 mg/ml) for 20 minutes and visualized under UV light using a GEL Doc™ imaging system. Fragment sizes were determined by comparing their migration to a 20 bp DNA ladder using Alpha-Ease FC 5.0 software (Alpha Innotech, USA).

Allele scoring was performed for the presence (1) or absence (0) of bands across all genotypes to create a binary matrix. Genetic diversity parameters, including the number of alleles per locus, major allele frequency, gene diversity, and polymorphic information content (PIC), were calculated using PowerMarker software version 3.25 (Liu and Muse, 2005). The binary data were analyzed using NTSYS-pc version 2.2 (Rohlf, 2002) to generate a UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram depicting genetic relationships among the genotypes.

## Results and Discussion

**Screening of rice genotypes for salinity tolerance at germination stage**

At germination stage, rice genotypes were categorized into 5 groups following the standard evaluation system (IRRI, 1996). The groups were chronologically highly tolerant, tolerant, moderately tolerant, susceptible and highly susceptible. For each group, a numerical value (score) in the scale of 1 to 9 was assigned (Table 3 and table 4). Under control conditions, the rice genotypes exhibited a high germination percentage, which declined under applied stress. In this experiment, a wide variation in stress tolerance was observed among the 25 genotypes screened. Twenty percent (5 of 25) were classified as highly tolerant and 40% (8 of 25) as tolerant, supporting the finding by Almansouri et al. (2001) that germination percentage alone does not always show clear and consistent differences across varieties under stress. Based on mean percent germination reduction, the genotypes Kakua, Sona joly, Dud shail, and Roa were identified as highly tolerant (score 1). The genotypes Gajor goria, Bina shail, Bogi, Sylhet balam, Ashi binni, Anik, Goccha, and Biran were classified as tolerant (score 3). This inhibitory effect of stress on germination, particularly from NaCl, is well established (Rajakumar, 2013) and is often a result of limited mobilization of energy reserves from the starchy endosperm (Hakim et al., 2010). Remarkably, unlike the vast majority of higher plants whose seeds fail to germinate without oxygen, rice retains a unique tolerance to anaerobiosis, capable of successful germination even under complete anoxia (Perata and Alpi, 1993).

**Screening of rice genotypes for stress tolerance at seedling stage**

All genotypes were grown robustly and showed uniform green color and height in the non-stressed condition. In salinized and submergence condition, the genotypes showed wide variation in phenotypes ranging from score 1 (highly tolerant) and score 9 (highly susceptible).

**Plant height**

Plant height, a key morphological parameter, was significantly affected by various stress conditions (Tables 5). Final plant height measurement revealed that under control conditions, values ranged from 22.2 cm (Binadhan 7) to 58.1 cm (Dud shail). All stress treatments reduced plant height across genotypes, with Binadhan 7 consistently exhibiting the shortest plants and Dud shail the tallest. Notably, the genotype Machranga demonstrated the highest tolerance, with the smallest mean percent reduction in plant height (10%). To quantify tolerance, genotypes were classified into five categories—highly tolerant (score 1), tolerant (3), moderately tolerant (5), susceptible (7), and highly susceptible (9)—following the standard evaluation system (SES) scoring concept from IRRI (1996). No genotype was classified as highly tolerant (score 1). Nine genotypes were identified as tolerant (score 3): Kakua, Sona joly, Sylhet balam, Binadhan 10, Dud shail, Anik, Binadhan 8, Machranga, and Biran. Eleven genotypes were moderately tolerant (score 5): Ashi binni, FL-378, FL-478, Nonia, Goccha, Talmuri, Agar, Roa, Baghnoli, Nazira shail, and Binadhan 11. Three genotypes were susceptible (score 7): Agar, Gajor goria, and Binadhan 7. Finally, two genotypes, Bina shail and Bogi, were highly susceptible (score 9).

This pattern of response is consistent with established principles. While submergence stress is known to promote shoot elongation in rice (Ismail et al., 2009), reduced elongation is a key advantage for survival during flash floods, as elongated seedlings are prone to lodging upon water recession (Suge, 1985; Setter and Laureles, 1996). Our results confirm previous reports that stem elongation is more pronounced in susceptible varieties than in tolerant ones (Singh et al., 2001; Das et al., 2005), indicating that limited elongation is a trait associated with submergence tolerance. Furthermore, the significant reduction in plant height and biomass in susceptible lines under stress aligns with findings in salt-stressed rice, where reductions were far more severe in susceptible lines compared to tolerant ones (Bhowmik et al., 2009).

**Table 3.** Stress induced changes in germination percentage

| Genotypes | No. of seed used for germination | % Germination under control | % Germination in saline condition | % Reduction in saline condition | Score |
|---|---|---|---|---|---|
| Agar | 100 | 75 | 52 | 32 | 7 |
| Gajor goria | 100 | 96 | 86 | 10 | 3 |
| Bina shail | 100 | 90 | 82 | 9 | 3 |
| Bogi | 100 | 97 | 90 | 7 | 3 |
| Kakua | 100 | 99 | 95 | 4 | 1 |
| FL-378 | 100 | 70 | 55 | 22 | 7 |
| Sona joly | 100 | 98 | 95 | 3 | 1 |
| Binadhan 7 | 100 | 95 | 75 | 21 | 7 |
| Nazira shail | 100 | 80 | 41 | 49 | 9 |
| Sylhet balam | 100 | 92 | 84 | 9 | 3 |
| Baghnoli | 100 | 78 | 46 | 41 | 9 |
| Ashi binni | 100 | 94 | 83 | 12 | 3 |
| Binadhan 10 | 100 | 98 | 90 | 8 | 3 |
| Talmuri | 100 | 80 | 50 | 38 | 7 |
| Dud shail | 100 | 95 | 90 | 5 | 1 |
| Anik | 100 | 95 | 87 | 9 | 3 |
| FL-478 | 100 | 80 | 50 | 38 | 9 |
| Boiri | 100 | 78 | 30 | 62 | 9 |
| Roa | 100 | 94 | 91 | 3 | 1 |
| Machranga | 100 | 85 | 50 | 41 | 9 |
| Nonia | 100 | 85 | 66 | 22 | 5 |
| Binadhan 8 | 100 | 99 | 95 | 4 | 1 |
| Goccha | 100 | 95 | 88 | 7 | 3 |
| Biran | 100 | 60 | 52 | 13 | 3 |
| Binadhan 11 | 100 | 99 | 92 | 7 | 3 |

**Table 4** Categories of rice genotypes based on germination percent as affected by salt stress

| Tolerance level | Rice genotypes |
|---|---|
| Highly tolerant (Score 1) | Kakua, Sona joly, Dud shail, Roa. Binadhan 8 = 5 genotypes |
| Tolerant (Score 3) | Gajor goria, Bina shail, Bogi, Sylhet balam, Ashi binni, Binadhan 10, Anik, Goccha, Biran, Binadhan 11 = 10 genotypes |
| Moderately tolerant (Score 5) | Nonia = 1 genotype |
| Susceptible (Score 7) | Agar, FL-378, Talmuri, Binadhan 7 = 4 genotypes |
| Highly susceptible (Score 9) | Nazira shail, Baghnoli, FL-478, Boiri, Machranga = 5 genotypes |

**Table 5** Stress induced changes in plant height at seedling stage

| Genotypes | Plant height under control (cm) | 8 dS/m salinity | | 12 dS/m Salinity | | submerged condition | | CSS | | Mean % reduction | Score |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | plant height (cm) | % reduction | plant height (cm) | % reduction | plant height (cm) | % reduction | plant height (cm) | % reduction | | |
| Agar | 30.1 | 26 | 14 | 20.2 | 33 | 25.1 | 17 | 20 | 34 | 24 | 7 |
| Gajor goria | 31.7 | 27.1 | 15 | 21 | 34 | 22.1 | 30 | 25 | 21 | 25 | 7 |
| Bina shail | 29.3 | 21.1 | 28 | 19 | 35 | 23.2 | 21 | 22 | 25 | 27 | 9 |
| Bogi | 45.1 | 35 | 22 | 31.4 | 30 | 30 | 34 | 25 | 45 | 33 | 9 |
| Kakua | 35.3 | 33.1 | 6 | 30.4 | 14 | 30.2 | 15 | 28 | 21 | 14 | 3 |
| FL-378 | 28.1 | 26 | 8 | 21.7 | 23 | 22.2 | 21 | 20.2 | 28 | 20 | 5 |
| Sona joly | 46.6 | 42.9 | 8 | 40 | 14 | 43 | 8 | 40 | 14 | 11 | 3 |
| Binadhan 7 | 22.2 | 18.6 | 16 | 18.2 | 18 | 16 | 28 | 15 | 33 | 24 | 7 |
| Nazira shail | 29.1 | 27 | 7 | 23.1 | 21 | 22 | 25 | 20.6 | 29 | 20 | 5 |
| Sylhetbalam | 45.1 | 42.2 | 7 | 40 | 11 | 40.3 | 11 | 38.1 | 16 | 11 | 3 |
| Baghnoli | 38.8 | 34.1 | 12 | 30.1 | 23 | 30.4 | 22 | 27 | 31 | 22 | 5 |
| Ashi binni | 40.3 | 35 | 13 | 32.1 | 20 | 30.1 | 25 | 28 | 31 | 22 | 5 |
| Binadhan10 | 34 | 31.5 | 7 | 30.2 | 11 | 28.7 | 16 | 27.1 | 20 | 14 | 3 |
| Talmuri | 48.5 | 42.1 | 13 | 40.4 | 17 | 41.4 | 15 | 40 | 18 | 16 | 5 |
| Dud shail | 58.1 | 52.4 | 10 | 50.1 | 14 | 51.8 | 11 | 50.1 | 14 | 12 | 3 |
| Anik | 45.3 | 41.5 | 8 | 39.2 | 14 | 40.2 | 11 | 39.7 | 12 | 11 | 3 |
| Fl-478 | 37.4 | 33.5 | 11 | 30 | 20 | 31.2 | 17 | 29.4 | 21 | 17 | 5 |
| Boiri | 40 | 35.8 | 11 | 32 | 20 | 31.4 | 22 | 20 | 50 | 26 | 5 |
| Roa | 39.2 | 33.3 | 15 | 30.7 | 22 | 32.5 | 17 | 31 | 21 | 19 | 5 |
| Machranga | 45.9 | 41.1 | 11 | 40 | 13 | 42.6 | 7 | 42 | 9 | 10 | 3 |
| Nonia | 38.3 | 32.4 | 16 | 29.3 | 24 | 32.1 | 16 | 31.2 | 19 | 19 | 5 |
| Binadhan 8 | 32.9 | 30.4 | 8 | 28.2 | 14 | 31.1 | 6 | 28.1 | 15 | 11 | 3 |
| Goccha | 38.6 | 34 | 12 | 31.1 | 20 | 32.4 | 16 | 30.1 | 22 | 17 | 5 |
| Biran | 47.3 | 42 | 11 | 38.5 | 19 | 42.3 | 11 | 39 | 18 | 15 | 3 |
| Binadhan11 | 30.3 | 28.2 | 7 | 25 | 18 | 24.2 | 20 | 21.2 | 30 | 19 | 5 |

**Fresh weight**

Similar to plant height, fresh weight was also significantly reduced by stress treatments. In control plants, fresh weight ranged from 0.47 g (Boiri) to 1.19 g (Gajor goria). All stress conditions decreased fresh weight, with Boiri and Kakua consistently showing the lowest values across various treatments. The genotype Kakua Binni exhibited the highest tolerance, with the smallest mean percent reduction (17%). Based on the percent decrease in fresh weight, genotypes were categorized into five tolerance classes and assigned scores, as detailed in Table 6. From the mean percent fresh weight reduction, no genotype got score 1 i.e. no genotype

was found as highly tolerant to stress. 9 genotypes got score 3 i.e. Kakua, Sona joly, Sylhet balam, Ashi binni, Machranga, Bogi, Biran, Baghnoli, Goccha were found tolerant to stress. 9 genotypes got the score 5 i.e Gajor goria, Bina shail, Nazira shail, Dud shail, FL-378, FL-478, Roa, Talmuri, Binadhan 8 were found moderately tolerant to stress. 3 genotypes got the score 7 i.e. Bogi, Ashi binni, Anik were found as susceptible to stress. 4 genotypes got the score 9 i.e. Agar, Boiri, Binadhan 7, Binadhan 11 were found as highly susceptible to stress. Complete submergence at the seedling stage causing a dramatic decline in grain yield in intolerant varieties (Singh et al., 2009).

**Table 6.** Stress induced changes in fresh weight in seedling stage

| Genotypes | Fresh weight under control (g) | 8 dS/m salinity | | 12 dS/m salinity | | submergence | | CSS | | Mean % reduction | Score |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fresh weight (g) | % reduction | Fresh weight (g) | % reduction | plant height (cm) | Fresh weight (g) | % reduction | Fresh weight (g) | | |
| Agar | 0.59 | 0.43 | 27 | 0.32 | 46 | 0.36 | 39 | 0.29 | 51 | 41 | 9 |
| Gajor goria | 1.19 | 1.01 | 15 | 0.78 | 35 | 0.96 | 19 | 0.76 | 36 | 26 | 5 |
| Bina shail | 0.75 | 0.63 | 16 | 0.42 | 44 | 0.67 | 11 | 0.41 | 45 | 29 | 5 |
| Bogi | 0.61 | 0.57 | 7 | 0.32 | 48 | 0.42 | 31 | 0.36 | 41 | 32 | 7 |
| Kakua | 1.1 | 0.96 | 123 | 0.9 | 18 | 0.91 | 17 | 0.87 | 21 | 17 | 3 |
| FL-378 | 0.76 | 0.62 | 19 | 0.48 | 37 | 0.6 | 21 | 0.53 | 30 | 27 | 5 |
| Sona joly | 0.58 | 0.51 | 12 | 0.47 | 19 | 0.49 | 16 | 0.4 | 31 | 20 | 3 |
| Binadhan 7 | 0.76 | 0.51 | 33 | 0.42 | 45 | 0.41 | 46 | 0.32 | 58 | 45 | 9 |
| Nazira shail | 0.69 | 0.56 | 19 | 0.47 | 32 | 0.53 | 23 | 0.32 | 54 | 32 | 5 |
| Sylhetbalam | 0.96 | 0.87 | 9 | 0.66 | 31 | 0.83 | 14 | 0.6 | 38 | 23 | 3 |
| Baghnoli | 0.57 | 0.5 | 12 | 0.41 | 28 | 0.49 | 14 | 0.32 | 44 | 25 | 3 |
| Ashi binni | 0.69 | 0.57 | 17 | 0.4 | 42 | 0.51 | 26 | 0.39 | 44 | 32 | 7 |
| Binadhan 10 | 0.96 | 0.83 | 14 | 0.67 | 30 | 0.8 | 17 | 0.61 | 37 | 24 | 3 |
| Talmuri | 0.56 | 0.49 | 13 | 0.32 | 43 | 0.45 | 20 | 0.3 | 47 | 30 | 5 |
| Dud shail | 0.78 | 0.63 | 19 | 0.51 | 35 | 0.58 | 26 | 0.47 | 40 | 30 | 5 |
| Anik | 0.57 | 0.45 | 21 | 0.37 | 35 | 0.41 | 28 | 0.31 | 46 | 33 | 7 |
| Fl-478 | 0.55 | 0.5 | 9 | 0.41 | 26 | 0.39 | 29 | 0.33 | 40 | 26 | 5 |
| Boiri | 0.47 | 0.32 | 32 | 0.29 | 38 | 0.3 | 36 | 0.21 | 55 | 41 | 9 |
| Roa | 0.67 | 0.56 | 17 | 0.4 | 40 | 0.51 | 24 | 0.4 | 40 | 30 | 5 |
| Machranga | 0.98 | 0.82 | 16 | 0.7 | 29 | 0.84 | 14 | 0.71 | 28 | 22 | 3 |
| Nonia | 0.74 | 0.61 | 18 | 0.56 | 24 | 0.6 | 19 | 0.53 | 28 | 22 | 3 |
| Binadhan 8 | 0.89 | 0.71 | 20 | 0.6 | 33 | 0.68 | 24 | 0.56 | 37 | 28 | 5 |
| Goccha | 0.76 | 0.56 | 26 | 0.41 | 46 | 0.52 | 32 | 0.4 | 47 | 38 | 7 |
| Biran | 0.53 | 0.4 | 25 | 0.32 | 40 | 0.41 | 23 | 0.3 | 43 | 33 | 7 |
| Binadhan11 | 0.96 | 0.71 | 26 | 0.64 | 33 | 0.5 | 48 | 0.42 | 56 | 41 | 9 |

**Dry weight**

Dry weight exhibited significant variation across rice genotypes and was notably reduced by all stress treatments. Under control conditions, dry weight ranged from 0.031 g (Talmuri) to 0.077 g (Binadhan 10). Stress treatments consistently decreased dry weight, with Talmuri (or Agar for CSS) yielding the lowest values and Binadhan 10 the highest. The genotype Kakua demonstrated the greatest tolerance, with the smallest mean percent reduction (18%). Genotypes were subsequently classified into five tolerance categories based on their percent decrease in dry weight, and scores were assigned as detailed in Table 7. Dry weight reduction under stress served as a key metric for assessing physiological tolerance. Based on the mean percent reduction, genotypes were categorized, revealing a strong susceptibility in the population. No genotype was classified as highly tolerant (score 1). Only one genotype, Kakua, was identified as tolerant (score 3). Six genotypes were moderately tolerant (score 5): Binadhan 10, Boiri, Bogi, Bina shail, Machranga,

and Binadhan 8. Nine genotypes were susceptible (score 7): Binadhan 11, Goccha, Anik, FL-478, Talmuri, Nazira shail, Sona joly, Binadhan 7, and Gajor goria. A further nine genotypes were highly susceptible (score 9): Agar, Ashi binni, Sylhet balam, Baghnoli, Biran, Roa, Nonia, Dud shail, and FL-378.

The widespread reduction in dry matter accumulation observed in this study is consistent with established findings in plant stress physiology. The decline in biomass can be largely attributed to impaired mobilization and depletion of carbohydrate reserves under stress conditions. Previous studies have demonstrated that submergence stress significantly reduces starch and total soluble sugar contents in seedlings (Palada and Vergara, 1972; Ram et al., 2001). Similarly, salinity stress has been reported to suppress starch accumulation (Downton, 1997). The pronounced reduction in dry weight recorded in most genotypes in the present experiment contrasts sharply with the growth optima observed in certain halophytic species (e.g., Clough, 1984; Burchett et al., 1989), thereby underscoring the high sensitivity of these rice varieties to the imposed salinity and submergence stresses.

**Table 7** Stress induced changes in Dry weight in seedling stage

| Genotypes | Dry weight under control (g) | 8 dS/m salinity | | 12 dS/m salinity | | Submergence | | CSS | | Mean % reduction | Score |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Dry weight (g) | % reduction | Dry weight (g) | % reduction | Dry weight (g) | % reduction | Dry weight (g) | % reduction | | |
| Agar | 0.05 | 0.04 | 20 | 0.034 | 32 | 0.021 | 96 | 0.011 | 78 | 56 | 9 |
| Gajor goria | 0.067 | 0.052 | 22 | 0.032 | 52 | 0.05 | 25 | 0.041 | 39 | 35 | 7 |
| Bina shail | 0.058 | 0.05 | 14 | 0.041 | 29 | 0.047 | 19 | 0.03 | 48 | 28 | 5 |
| Bogi | 0.056 | 0.05 | 11 | 0.032 | 43 | 0.048 | 14 | 0.031 | 45 | 28 | 5 |
| Kakua | 0.04 | 0.037 | 8 | 0.031 | 23 | 0.032 | 20 | 0.031 | 23 | 18 | 3 |
| FL-378 | 0.05 | 0.037 | 26 | 0.029 | 42 | 0.031 | 38 | 0.022 | 56 | 41 | 9 |
| Sona joly | 0.041 | 0.03 | 27 | 0.025 | 39 | 0.031 | 24 | 0.019 | 54 | 36 | 7 |
| Binadhan 7 | 0.07 | 0.054 | 23 | 0.04 | 43 | 0.05 | 29 | 0.031 | 56 | 38 | 7 |
| Nazira shail | 0.051 | 0.043 | 16 | 0.031 | 39 | 0.035 | 31 | 0.021 | 59 | 36 | 7 |
| Sylhetbalam | 0.057 | 0.04 | 30 | 0.032 | 44 | 0.037 | 35 | 0.022 | 61 | 43 | 9 |
| Baghnoli | 0.042 | 0.031 | 26 | 0.023 | 45 | 0.029 | 31 | 0.019 | 55 | 39 | 9 |
| Ashi binni | 0.076 | 0.054 | 29 | 0.033 | 57 | 0.035 | 54 | 0.025 | 67 | 52 | 9 |
| Binadhan 10 | 0.077 | 0.069 | 10 | 0.05 | 35 | 0.061 | 21 | 0.042 | 45 | 28 | 5 |
| Talmuri | 0.031 | 0.026 | 16 | 0.017 | 45 | 0.021 | 32 | 0.012 | 61 | 39 | 7 |
| Dud shail | 0.071 | 0.055 | 23 | 0.04 | 44 | 0.045 | 37 | 0.034 | 52 | 39 | 9 |
| Anik | 0.032 | 0.026 | 19 | 0.019 | 41 | 0.023 | 28 | 0.014 | 56 | 36 | 7 |
| FL-478 | 0.035 | 0.027 | 23 | 0.02 | 43 | 0.025 | 29 | 0.017 | 51 | 36 | 7 |
| Boiri | 0.025 | 0.02 | 20 | 0.018 | 28 | 0.019 | 24 | 0.012 | 52 | 31 | 5 |
| Roa | 0.054 | 0.041 | 24 | 0.03 | 44 | 0.035 | 35 | 0.025 | 54 | 39 | 9 |
| Machranga | 0.078 | 0.069 | 12 | 0.052 | 33 | 0.057 | 27 | 0.04 | 49 | 30 | 5 |
| Nonia | 0.05 | 0.041 | 18 | 0.029 | 42 | 0.03 | 40 | 0.024 | 52 | 38 | 9 |
| Binadhan 8 | 0.065 | 0.058 | 11 | 0.041 | 37 | 0.05 | 23 | 0.038 | 42 | 28 | 5 |
| Goccha | 0.045 | 0.037 | 18 | 0.029 | 36 | 0.031 | 31 | 0.023 | 49 | 33 | 7 |
| Biran | 0.032 | 0.027 | 16 | 0.016 | 50 | 0.02 | 38 | 0.012 | 63 | 41 | 9 |
| Binadhan 11 | 0.077 | 0.06 | 22 | 0.051 | 34 | 0.05 | 35 | 0.032 | 59 | 23 | 7 |

**Mean Stress tolerance score (MSTS)**

The mean stress tolerance score (MSTS) was computed for each genotype by averaging the individual tolerance scores from all measured growth parameters. Among the 25 genotypes evaluated, Kakua was the sole genotype to achieve an MSTS of 3, classifying it as tolerant to combined salinity and submergence stress (Table 8). The SES score, which provides an integrated measure of visual stress injury, revealed a broad spectrum of tolerance among the genotypes. A single genotype, Kakua, was classified as highly tolerant

(score 1). Four genotypes were identified as tolerant (score 3): Sona joly, FL-478, Boiri, and Biran. The largest group, comprising 13 genotypes, was rated as moderately tolerant (score 5): Binadhan 10, Nazira shail, Sylhet balam, Talmuri, Dud shail, Anik, Nonia, Bogi, Bina shail, Gajor goria, Binadhan 11, Binadhan 8, and Goccha. Five genotypes were susceptible (score 7): Binadhan 7, Baghnoli, Ashi binni, Roa, and Machranga. Finally, one genotype, FL-378, was highly susceptible (score 9).

This pattern of response, where only a few genotypes show strong tolerance and the majority exhibit moderate to high susceptibility, aligns with the expected synergistic effect of combined stresses. As established in the literature, combined flooding and salinity typically decrease growth and survival more severely than either stress alone (Van der Moezel et al., 1988, 1991; Marcar et al., 1993; Noble and Rogers, 1994). The significant differences in injury rates observed among our cultivars are consistent with the findings of Hamed et al. (2013), confirming that visual scoring based on SES is a reliable method for screening and identifying resistant germplasm under such complex stress conditions.

**Table 8** Mean stress tolerance score (SES) of different growth parameters of tested rice genotypes calculated for stress at seedling stage

| Rice genotypes | Mean SES | Plant height | Fresh weight | Dry weight | Mean score |
|---|---|---|---|---|---|
| Agar | 7 | 7 | 9 | 9 | (8) = 7 |
| Gajor goria | 5 | 7 | 5 | 9 | (6.5) = 7 |
| Bina shail | 5 | 9 | 5 | 7 | (6.5) = 7 |
| Bogi | 5 | 9 | 7 | 5 | (6.5) =7 |
| Kakua | 1 | 3 | 3 | 5 | 3 |
| FL-378 | 9 | 5 | 5 | 3 | (5.5) = 5 |
| Sona joly | 3 | 3 | 3 | 9 | (4.5) = 5 |
| Binadhan 7 | 7 | 7 | 9 | 7 | (7.5) = 7 |
| Nazira shail | 5 | 5 | 5 | 7 | (5.5) = 5 |
| Sylhet balam | 5 | 3 | 3 | 7 | (4.5) = 5 |
| Baghnoli | 7 | 5 | 3 | 9 | (6) = 5 |
| Ashi binni | 7 | 5 | 7 | 9 | 7 |
| Binadhan 10 | 5 | 3 | 3 | 9 | 5 |
| Talmuri | 5 | 5 | 5 | 5 | 5 |
| Dud shail | 5 | 3 | 5 | 7 | 5 |
| Anik | 5 | 3 | 7 | 9 | (6) = 5 |
| FL-478 | 3 | 5 | 5 | 7 | 5 |
| Boiri | 3 | 5 | 9 | 7 | (6) = 5 |
| Roa | 7 | 5 | 5 | 5 | (5.5) = 5 |
| Machranga | 7 | 3 | 3 | 9 | (5.5) = 5 |
| Nonia | 5 | 5 | 3 | 5 | (4.5) = 5 |
| Binadhan 8 | 5 | 3 | 5 | 9 | (5.5) = 5 |
| Goccha | 5 | 5 | 7 | 5 | (5.5) = 5 |
| Biran | 3 | 3 | 7 | 7 | 5 |
| Binadhan 11 | 5 | 5 | 9 | 9 | 7 |

**Screening with SSR markers**

The evaluation of genetic diversity and similarity is crucial for rice improvement, as well as for the efficient management and conservation of germplasm resources. This was achieved through DNA fingerprinting using Simple Sequence Repeat (SSR), or microsatellite markers. Microsatellite-enriched DNA fingerprints were constructed using standard procedures. In this study, 25 rice germplasms were analyzed using two primers, RM585 and SC3, selected for their relevance to salinity and submergence tolerance. The bands generated for each genotype were compared against those of known salinity-tolerant checks (Binadhan 8, Binadhan 10, and FL-478) and a submergence-tolerant check (Binadhan 11). Amplified microsatellite loci were resolved via Polyacrylamide Gel Electrophoresis (PAGE) to assess polymorphism. The microsatellite profiles for the 25 rice germplasms at loci RM585 and SC3 are shown in Figure 1 and 2, respectively.

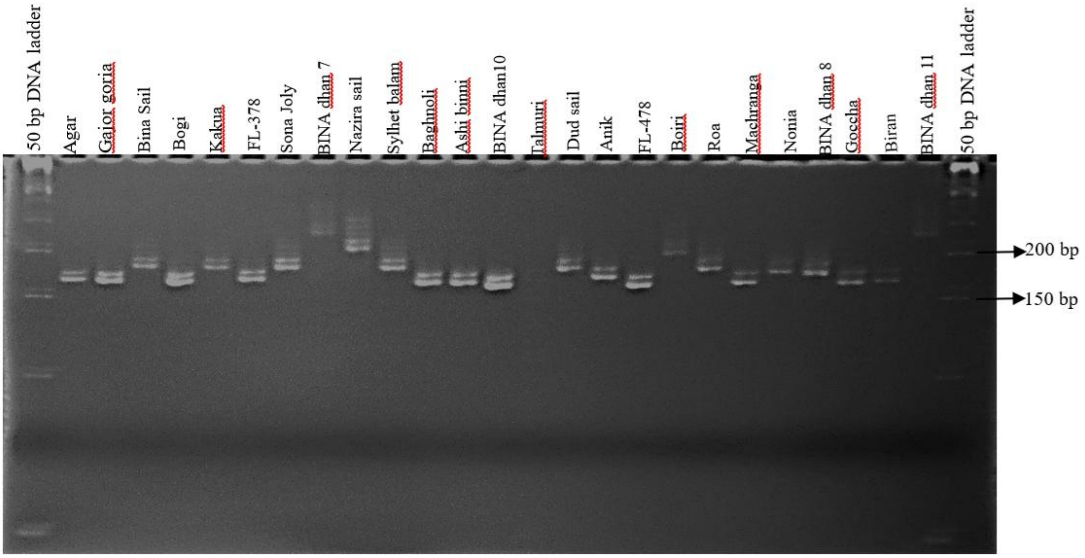


**Figure 1.** Microsatellite profiles of 25 rice genotypes at loci RM585

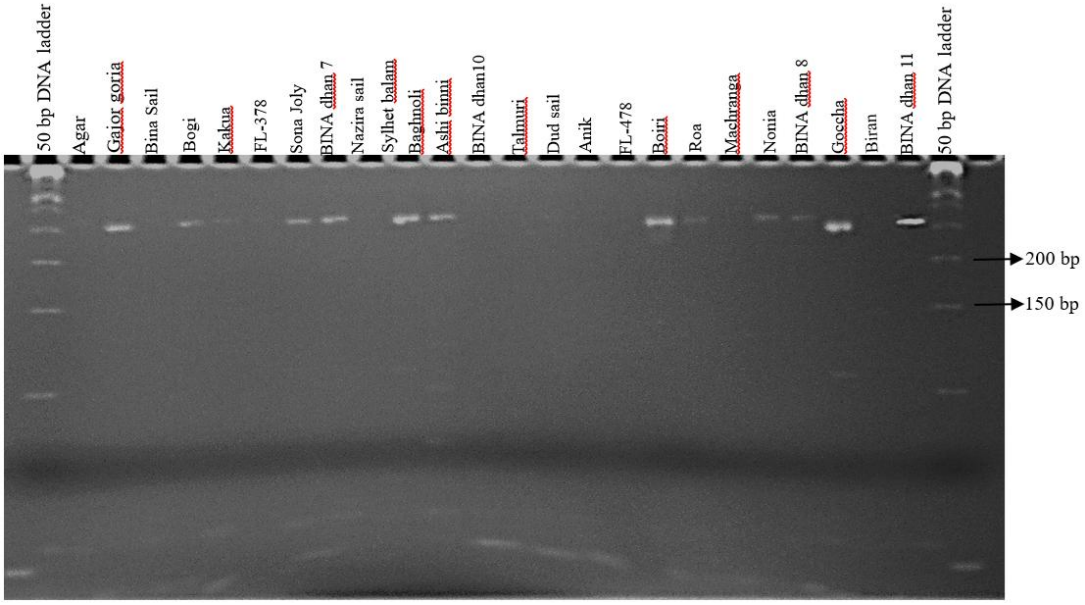


**Figure 2.** Microsatellite profile of 25 rice genotypes at loci SC3

The results revealed that both primers detected polymorphism among the analyzed rice germplasm. The microsatellite loci were also found to be multi-allelic, with 7 to 13 alleles per locus and a mean of 10 alleles per locus in this study.

**Molecular Characterization and Genetic Diversity Analysis**

Twenty-five rice genotypes were characterized using two microsatellite (SSR) markers. The major allele frequency, number of alleles, gene diversity, and polymorphic information content (PIC) for each marker were calculated using PowerMarker software. Both markers exhibited high polymorphism, revealing a total of 20 alleles with an average of 10 alleles per locus, ranging from 7 to 13. This high allele count is a key criterion for assessing a marker's utility in diversity studies, as it enhances the ability to discriminate between genetically similar genotypes (Seetharam et al., 2009). Our results are consistent with findings by Dhar et al. (2012), who reported a similar average number of alleles per locus. The mean gene diversity for the marker set was 0.787 (Table 9), indicating a high probability (78.7%) that two randomly selected alleles would be different. Values for individual loci ranged from 0.720 (SC3) to 0.854 (RM585). Gene diversity was significantly correlated with the number of alleles, repeat motif, and allele size range, which aligns with previous research (Heenan et al., 2000). The polymorphic information content (PIC) value further confirmed the utility of these markers. PIC reflects both the number of alleles and the evenness of their distribution. A higher PIC value denotes a more informative marker. Both markers exceeded the 0.5 threshold for informative markers (Jiang et al., 2010), with RM585 and SC3 having PIC values of 0.841 and 0.678, respectively.

**Table 9** Size and frequency of alleles and diversity index at 2 SSR loci across 25 rice varieties

| Locus | Major allele | | Genotype No. | No. of alleles | Rare alleles | Null allele | PIC | Gene Diversity | SMM Index |
|---|---|---|---|---|---|---|---|---|---|
| | Size (bp) | Freq. (%) | | | | | | | |
| RM585 | 191 | 0.280 | 13.0 | 13.0 | 9.0 | 2.0 | 0.841 | 0.854 | 0.00 |
| SC3 | 176 | 0.400 | 7.00 | 7.00 | 3.0 | 2.0 | 0.678 | 0.720 | 0.00 |
| Total | 367 | 0.680 | 20.0 | 20.0 | 12.0 | 4.0 | 1.519 | 1.574 | 0.00 |
| Mean | 183.5 | 0.34 | 10.0 | 10.0 | 6.00 | 2.0 | 0.760 | 0.787 | 0.00 |

**Cluster analysis and correlation with phenotypic data**

A dendrogram (Figure 3) constructed from the polymorphism data grouped the 25 genotypes into six clusters. Cluster VI contained eight genotypes, most of which were phenotypically classified as tolerant or moderately tolerant based on SES scores. Notable exceptions in this cluster were the highly tolerant genotype Kakua and the susceptible genotype Roa. This cluster also included the known stress-tolerant check variety Binadhan 8 and was phylogenetically proximate to Cluster V, which contained the submergence-tolerant check variety Binadhan 11.

The results indicate that selection for tolerance to combined salinity and submergence stress at the germination stage may not be highly reliable. Screening based on agronomic parameters at the seedling stage proved more dependable, as most genotypes identified as tolerant at that stage were corroborated by molecular clustering. An exception was genotype Binadhan 7, which was phenotypically susceptible yet grouped in the same cluster as the tolerant variety Binadhan 11, suggesting a genetic potential for tolerance not expressed under the applied stress conditions or a divergence between genetic relatedness and phenotypic performance.

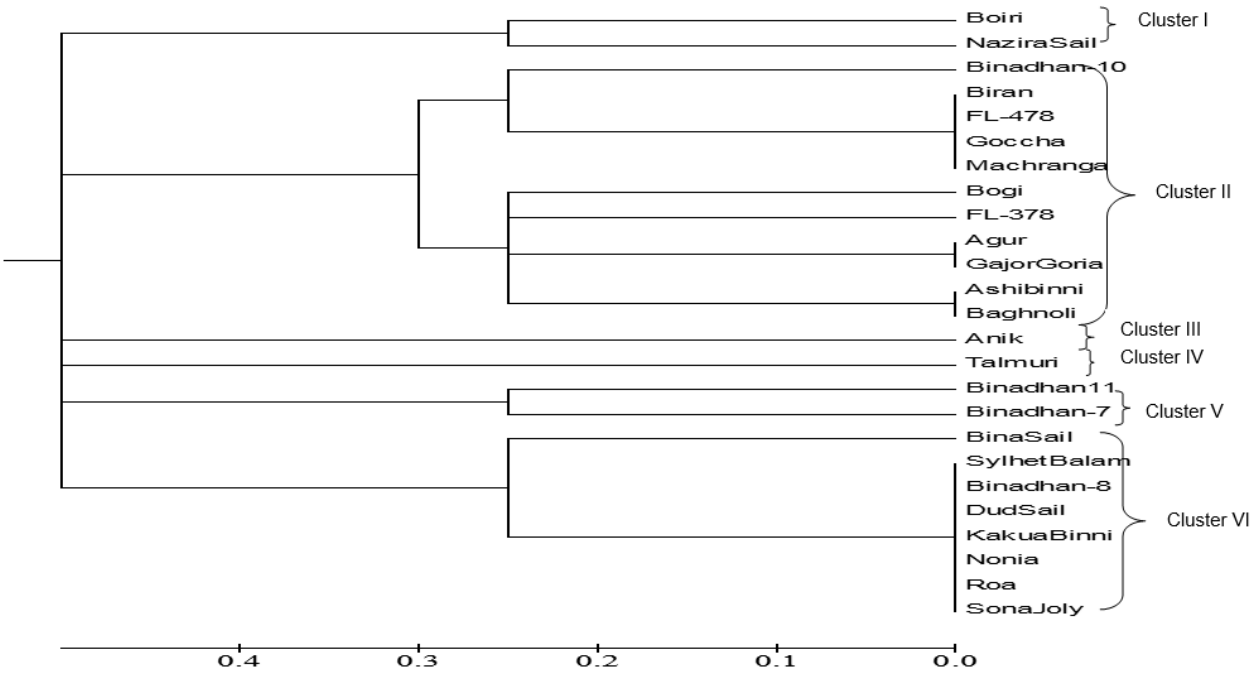


**Figure 3.** UPGMA Dendrogram based on Nei's (1972) genetic distance, summarizing data on differentiation among 25 rice genotypes according to SSR analyses (Sub cluster was cut at 50% of average Nei's genetic distance 0.390)

## Conclusion

This study demonstrates that combined salinity and submergence stress significantly impairs germination and seedling growth in rice, yet reveals substantial genetic variation in tolerance among the genotypes. Phenotypic screening identified several promising genotypes, with the landrace Kakua exhibiting superior tolerance, as evidenced by the lowest reductions in germination (7%) and dry weight (13%), and a favorable stress tolerance score. Sona joly also showed notable resilience. Genotypic analysis using SSR markers (RM585 and SC3) confirmed a high degree of genetic diversity among the 25 genotypes, with an average of 10 alleles per locus and mean Polymorphic Information Content (PIC) of 0.760. The cluster analysis successfully distinguished the landraces and grouped known tolerant check varieties (e.g., salt-tolerant Binadhan10 in Cluster II and submergence-tolerant genotypes in Cluster VI, validating the molecular markers' effectiveness. In summary, the landrace Kakua emerges as a prime candidate for future breeding programs aimed at enhancing tolerance to combined salinity and submergence stress. The correlation between phenotypic performance and genetic clustering provides a strong foundation for the marker-assisted selection of these traits.

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## Conflict of Interest

There are no conflicts of interest declared by the authors.

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