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**Original Research Article**

# Phenotypic and Proteomic Responses of Rice to Complete Submergence

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**A B S T R A C T**

The present study was conducted to evaluate 13 rice genotypes for submergence tolerance in rice. Tolerant to submergence stress is an important breeding objective in areas where rice cultivars are subjected to complete inundation for a week or more. Submergence stress was evaluated at vegetative stage in pot experiment. At vegetative stage, pots having 40 days old seedlings were submerged in pond. During submergence period, water level was maintained at 1.10 m. The level of pond water was maintained through water pipe. Control was kept without any submergence. After 14 days of submergence most of the rice genotypes died except Swarna sub 1, NDR 98030144 and IR64, which survived with 4, 2 and 1 tillers, respectively. *Swarna Sub1*, a highly submergence tolerance rice cultivar and *Swarna*, a high-yielding and widely adapted, submergence-susceptible rice variety were subjected to SDS –PAGE protein profiling. The result confirms the presence of novel band in the treated sample of *Swarna Sub1* variety. The SDS-PAGE results showed that *Swarna Sub 1* had one noble protein band of 26 kDa in comparison to *Swarna* under submerged sample. The genome-wide proteomic comparison between tolerant *Swarna Sub1 (Sub1)* and sensitive *Swarna* after submergence provides insight into the transcriptional regulation of the Sub1 mediated tolerance response. These findings establish the foundations of introducing submergence tolerance into agriculturally desirable cultivars of rice.

## Introduction

Rice (*Oryza sativa* L.) is the staple food of about 50% of the world population. The production of rice is greatly hampered due to some abiotic stresses like salinity, drought, and submergence. In contrast to other crop species, rice is well known for its ability to grow in flooded soil. Rice is one of the few crop species that can germinate and grow in permanently waterlogged soils. Submergence of rice (*Oryza sativa*) by flash flooding is a major constraint to rice production in Asia. The environments of rainfed lowland rice are highly variable both over time and location. Flash-flooding and submergence adversely affect at least 16% of the rice lands of the world (22 m ha). In eastern India, 13 m ha of rice lands are unfavourably affected by excess water and periodically suffer from flash-floods and complete submergence. Improvement of germplasm is likely the best option to withstand submergence and stabilize productivity in these environments (*Septiningsih, et al., 2009*)

Here, the term "flooding" is used to describe the inundation by water of all or part of a plant. We use "water logging" to describe flooding of the root system and "submergence" to describe the situation when most or all aerial tissue is under water. Flood water can be fresh, stagnant, or saline and affect a plant once or multiple times in a growing season. These inundation events can result from flash floods, seasonal rises in surface water at low elevations, or tidal surges. The sudden saturation of well-drained soil causes rhizosphere microbes to rapidly consume available oxygen, triggering changes in the soil ecosystem that can alter the fixation of nitrogen and the availability of other nutrients to the plant. Floods are often accompanied by a decrease in soil pH, which increases the solubility of toxic metals, including iron and manganese, as well as phosphorus and other elements (*Setter et al., 2009*).

Accordingly, submergence tolerance of rice is an important trait for agricultural productivity. Many genes regulated during submergence are suspected to be involved in the physiological mechanisms of submergence tolerance of rice. Among these, *OsEXP* (*Huang et al., 2000; Cho and Kende, 1997*), *OsUSP* (*Sauter et al., 2002*), and *OsDD3*, were reported to have specific functions as cell expansion, universal stress protein, and putative type 1A plasma membrane receptor, respectively. In addition to *OsARP* (*O. sativa* antiporter regulating protein), two other submergence related genes *OsMGD* (*O. sativa* mono-galactosyl diacylglycerol synthase) and *OsGGT* (*O. sativa* glycogenin glucosyl transferase) were isolated from the submergence tolerant cultivar FR13A (*Qi et al., 2005a, b*). The present study was undertaken with the objective to evaluate rice genotypes under submerged condition and find out the novel protein with SDS-PAGE analysis.

## Materials and Methods

### Submergence evaluation

The submergence treatment was carried out at in newly constructed submergence pond adjoining to betwa drain at Crop Physiology experimental site in NDUAT, Kumarganj, Faizabad during *Kharif* season 2012-13. After 15 days of transplanting submergence tanks was completely filled up by water. Water depth was measured regularly and maintained by fresh water till 14$^{th}$ days of submergence. After 15$^{th}$ days of submergence treatment, water drained out from tanks.

### Observation recorded for growth in submergence condition

During submergence: growth observation were recorded at four stages of crop growth just before planting, before submergence, after de-submergence and at recovery stage. Three plants per replication were initially tagged for growth observations which were recorded over three replications. *(SES 1996)*

### Plant height

The plant height (cm) was measured from the base of stem i.e. the surface of ground up to the top of the panicle. The average height was calculated over three replications consisting three plants.

### Tillers per plant

Number of tillers per plant under each treatment was recorded by visual counting at different stage.

### Survival percentage

Plant survival percentage was recorded after 7 days of de-submergence. Survival was

indicated by the capacity of the plants to produce new leaves.

The differences between the number of plants/plot before submergence and number of plants/plot after 7 days of de-submergence indicates survival patterns.

### *Regeneration*

The total regenerated plants were counted at recovery stage *i.e*. after 15 days of de-submergence.

### *New leaf emergence*

The new leaves emergence was counted at recovery stage *i.e*. after 15 days of de-submergence.

## Protein profiling of rice leaf

### Reagent

All chemicals were of AR grade. Sodium phosphate buffer (0.25 M, pH 7.0), containing 0.15N NaCl, rice leaves, pestle and mortar, ice, centrifuge tubes etc.

### *Method*

The rice leaf protein was isolated as method described by *Laemmli, et. al, 1970.*

The fresh rice leaf were cut into small pieces using razor and crushed in sodium phosphate buffer (0.25M, pH 7.0) containing 0.15 NaCl.

It was homogenized mechanically and centrifuged at 10,000 g at $4^0$C for 20 minutes. This process was done twice.

After centrifugation the supernatant was collected. This supernatant was crude rice leaf protein for SDS –PAGE analysis.

## Gel electrophoresis of rice leaf protein

### Apparatus

Vertical slab gel type electrophoresis unit (Glass plate 18 x 9 x 0.1 cm) including power pack.

### Reagents

### Stock Acrylamide solution:

Acrylamide (30 %) - 29.20 g

Bis-acrylamide - 0.8 g

Double distilled water - 100 ml

### Separating gel buffer

1.875 M Tris HCl - 22.7 g (for 100 ml) pH 8.8

### Stacking Gel Buffers

0.6 M Tris HCl - 7.26 g (for 100 ml) pH 6.8

### Polymerizing Agent

Ammonium per Sulphate (10 %)
TEMED

### Electrode Buffer (pH 8.2)

0.025 M Tris HCl - 3.0 g
0.192 M Glycine - 14.5 g

0.1 % SDS - 1.0 g

Volume of Electrode buffer was made 1 L with double distilled water and adjusted to pH 8.2-8.3.

### SDS 10 %

1g in 10 ml double distilled water.

### Sample Extraction Buffer

0.25 M Sodium phosphate buffer (pH 7.0) containing 0.15N NaCl

### Loading dye (pH 6.8)

Tris HCl (pH 6.8, 1.5M) - 5 ml
SDS (10%) - 6ml
β-merceptoethanol - 1.5 ml
Bromophenol blue (1 %) - 100mg
Glycerol (15 %) - 15ml

Dissolve the above reagent and make total volume 100 ml with double distilled water and store it at $4^0$C.

### Gel staining solution (Coomassie brilliant blue R-250)

0.25 g Coomassie brilliant blue R-250 taken in 40 ml of methanol, to this 7 ml acetic acid added.

Made the final volume 100 ml with double distilled water (Prepared fresh before use)

### Destaining solution

Destaining solution was prepared by adding 10% methanol and 5% acetic acid.

Final volume was made 100 ml with double distilled water.

### Preparation of sample

The extracted rice leaf protein in phosphate buffer saline (0.25 M pH 7.0) containing 0.15N NaCl were used as a loading sample.

Heat the sample by mixing the sample and running dye in (60:40 ratio) for 5-10 minute for proper denaturation and the sample was centrifuged at 10000 g for 10 minutes at 20±2 $^0$C.

### Running gel solution

The polyacrylamide gel was prepared by the following method. The following solution was mixed serially as described in table 1. After adding TEMED and APS, gel will polymerize fairly and quickly, so do not add these reagent until it was ready to pour.

The stacking gel were put between glass plates up to proper mark and wait for 30- 40 minutes for proper polymerization of gel. The separating gel was cast, then inserted the Teflon comb (13 well) in the gap between the glass plates and waited for proper polymerization of the separating gel. After proper polymerization, the Teflon comb was carefully removed from the gel and plates were assembled into electrophoresis unit and electrode buffer was filled both in lower and upper tank of electrophoresis unit. After this the electrophoresis unit was attached with power pack and placed the gel for 8-10 hours with a supply of 25 mA and 160 volt current. When the tracking dye reached the end of the running gel after complete separation of protein molecules, power supply turned off. The gel was gently removed from the space between the plates, immersed in staining solution contained in a tray for proper staining.

### Destaining of gel

Destaining solution was prepared by adding 10% methanol and 5% acetic acid. Final volume made 100 ml with double distilled water. Putting it into the de-staining solution the processes was continued until the back ground of gel became colorless.

### Experimental materials

The material for this study consisted of 13 rice cultivars as given in table 2.

**Table.1** Separating and stacking gel solution

| **Separating Gel Solution** | | |
|---|---|---|
| **Solutions** | **12%** | **15 %** |
| $H_2O$ | 10.2 ml | 7.2 ml |
| 1.875M Tris-HCl pH 8.8 | 7.5 ml | 7.5 ml |
| 10 % SDS | 0.15 ml | 0.15 ml |
| Acrylamide, Bis-acrylamide (30 %) | 12 ml | 15 ml |
| 10% Ammonium per Sulphate | 0.15 ml | 0.15 ml |
| TEMED | 0.02 ml | 0.02 ml |
| Total volume | 30 ml | 30 ml |

| **Stacking Gel Solution** | |
|---|---|
| **Solution** | **4%** |
| $H_2O$ | 3.45 ml |
| 0.6 M Tris-HCl, pH 6.8 | 0.63 ml |
| 10 % (w/v) SDS | 0.10 ml |
| Acrylamide/Bis-acrylamide (30 % /0.8 % w/v) | 0.83 ml |
| 10% (w/v) Ammonium per Sulfate (APS) | 0.005 ml |
| TEMED | 0.005 ml |
| Total volume | 5.00 ml |

**Table.2** Name of the entries and their place of origin

| **S. No.** | **Name of Variety/Line** | **Origin Place** |
|---|---|---|
| **1.** | Swana | CRRI Cuttack |
| **2.** | Swarna Sub1 | IRRI Philippines |
| **3.** | Jal Priya | NDUAT |
| **4.** | Jal Lahri | NDUAT |
| **5.** | Jal Nidhi | NDUAT |
| **6.** | NDR9830111 | NDUAT Screening based line |
| **7.** | NDR98030132 | NDUAT Screening based line |
| **8.** | NDR98030135 | NDUAT Screening based line |
| **9.** | NDR98030144 | NDUAT Screening based line |
| **10.** | NDR9730018 | NDUAT Screening based line |
| **11.** | Sarjoo52 | NDUAT |
| **12.** | NDR359 | NDUAT |
| **13.** | IR64 | IRRI Philippines |

**Table.3** Submergence treated results of rice varieties

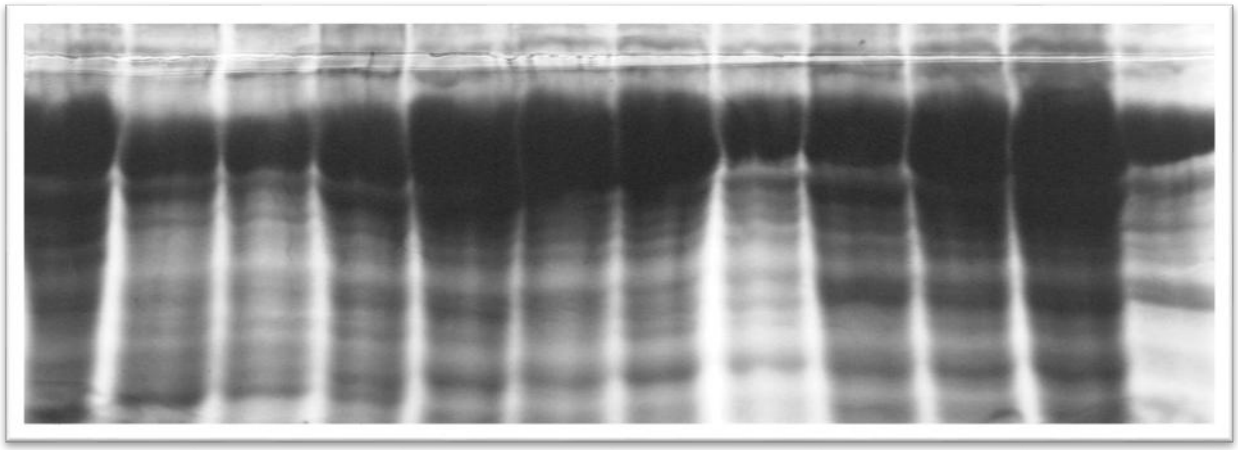
| Genotype / Lines | Plant height | No. of tillers | | | Plant height |
|---|---|---|---|---|---|
| | Before submergence | Before treatment | After treatment | Newly emerged tillers after submergence | Plant height of newly emerged tillers (cm) |
| Swarna | 76 | 14 | 1 | 0 | 0 |
| Swarna sub 1 | 52 | 12 | 4 | 5 | 24 |
| Jal Priya | 92 | 7 | 0 | 0 | 0 |
| Jal Lahri | 90 | 8 | 0 | 0 | 0 |
| Jal Nidhi | 86 | 6 | 0 | 0 | 0 |
| NDR98030111 | 75 | 7 | 0 | 0 | 0 |
| NDR98030132 | 79 | 7 | 0 | 0 | 0 |
| NDR98030135 | 74 | 8 | 0 | 0 | 0 |
| NDR98030144 | 72 | 8 | 2 | 3 | 27 |
| NDR9730018 | 68 | 6 | 0 | 0 | 0 |
| NDR 359 | 58 | 10 | 0 | 0 | 0 |
| Sarjoo-52 | 58 | 10 | 0 | 0 | 0 |
| IR 64 | 60 | 8 | 1 | 1 | 20 |

**Fig.1** Submergence treated pot

**Fig.2** After 14 days of submergence stress, three lines were regenerate

Swarna Sub1 NDR 98030144 IR 64

**Fig.3** SDS-PAGE of rice varieties in normal and submergence condition



*Swarna sub 1, NDR 98030144, NDR 98030111, NDR 98030132, NDR98030135, NDR9730018 (2 lane for each variety Ist for non-submerged and 2nd for submerged condiion).*

## Results and Discussion

### Phenotyping of rice genotypes under submergence condition

Rice genotypes were grown and maintained for 40 days (nursery days 20 + establishment days 20) in pots. Forty days old plant submerged in tank for 14 days and data were recorded on plant height and number of tillers before submergence. After submergence, newly emerged tillers and their height were recorded on 21 days. Plant height ranged from 52 (Swarna sub1) to 92 cm (Jalpriya). Maximum plant height was recorded Jalpriya (92 cm) followed by Jallahri (90cm), Jalnidhi (86 cm), NDR 98030132 (79 cm) and NDR98030111 (75cm). Number of tillers before submergence ranged from 6 (Jalnidhi) to 14 (NDR 9730018). After 14 days of submergence most of the rice genotypes died except Swarna sub 1, NDR 98030144 and IR64 (Fig 1& 2) which survived with 4, 2 and 1 tillers, respectively (Table 3). Result showed the beneficial effect of reduced

elongation growth on submergence tolerance of rice (*Setter and Laureles*, 1996). For submergence tolerance medium plant height with no stem elongation characteristics preferred as these genotype maintain their food (CHO) for regeneration ability after removal of submergence and this result was supported by *Kawano et al.,* (2009).

After 21 days from removal of stress Swarna sub1 and NDR98030144 exhibited regeneration ability with one more shoot, while IR 64 did not showed regeneration ability. The plant height, 24 cm was recorded for Swarna sub1, 27 cm for NDR98030144 and 20 cm for IR64 after 21days of de-submergence.

## Proteomic analysis of rice leaf protein

The SDS-PAGE results have been presented in Fig 3 for rice genotypes before submergence and after submergence. Results of Fig 3 indicated no significant differences among rice genotypes except *Swarna Sub 1* and NDR 98030144 which lacks one protein band under non-submergence condition. Results of (Fig 3) showed that *Swarna Sub 1* and NDR98030144 had one novel protein band under submerged sample as compared to non-submerged. This band needs its quantification and 'N' terminal sequencing.

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