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In-Vitro Screening for Salt Tolerance in Rice.

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ABSTRACT

Abiotic stresses are realized as the major yield reducing constraints in plants worldwide. Millions of hectares in Asia are unsuitable to cultivation due to salinity. Salt stress can affect crops in varied manner. Soil salinity > 8 dS/m or pH ≥ 9.8 is considered as high stress condition for a rice plant. Breeding salt tolerant varieties is considered most promising when compared to soil amelioration. Plant tissue culture has served as a tool in screening for abiotic resistance, especially in rice. The article attempts to review the efforts undertaken by plant breeders in screening for in vitro salt tolerance in rice crop.

Keywords – Rice, salt, tolerance, calli

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INTRODUCTION

Rice is synonymous to food in Asia, a life giving cereal, highly respected and considered auspicious by many in the world. It is also well known for its easy digestibility, taste and nutritive value. Varied forms of rice grains are preferred around the globe which range from bold to slender ones, small to extra-long grains, sticky to non sticky ones, polished white to unpolished red rice, non aromatic to aromatic, wild rice to hybrid rice, natural to fortified rice, etc.,

More than 90% of the world's rice is grown and consumed in Asia where 60% of the earth's people and about two-thirds of the poor live [1]. Large areas of land suitable for growing rice remain unplanted because of severe nutritional deficiencies and toxicities. A vast majority of rice soils are underutilized due to varying levels of alkalinity or salinity. Land area under cultivation of food crops is hence shrinking. Abiotic and biotic stresses are major hindrances in improving productivity. Inland salinity and coastal salinity are one of the major stress factors encountered in rice growing.

Salt tolerance is a complex trait that is controlled by multiple genes and involves various biochemical and physiological mechanisms. The functions of the distinct sets of genes involved in specific biochemical and physiological mechanisms must be combined to achieve substantial increases in salt tolerance [2]. Hence breeding for salt stress tolerance is considered important and many rice genotypes possessing tolerance have been released in India [3-6]. The International Rice Research Institute (IRRI) is in the path of developing the world's highest salt tolerant rice variety. They have succeeded in crossing the exotic wild rice species *Oryza coarctata* with IRRI rice variety IR56, a cultivated rice species of *Oryza sativa.*

Identification of salinity based markers will definitely help in introgressing salt tolerance in plants. QTLs for Na^+ and K^+ uptake of the shoots and roots controlling rice salt tolerance have been studied [7]. A rice quantitative trait locus for salt tolerance encoding a sodium transporter was studied by [8]. A major quantitative trait locus (QTL) for salt tolerance named Saltol was mapped on chromosome 1 [9]. *I*dentification of functional polymorphism for salt tolerance genes in rice has been carried out [10].

Transgenics with enhanced expression of vacuolar pyrophosphate pumps and H^+/Na^+ antiporters have been attempted for efficient storage of Na^+ and Cl^- ions inside the vacuole [11-15].

In vitro screening for salt stress

Plant cell and tissue culture techniques offer a potent tool in developing salt tolerant lines [16]. *In vitro* screening for salt tolerance can serve as a quick technique in preliminary screening of genotypes. Embryogenic calli derived by culturing in Murashige and Skoog (MS) media supplemented with 2,4-Dichlorophenoxyacetic acid (2,4-D) with or without the addition of cytokinins and additives have been utilised to screen for salt tolerance. Fresh or dry weight of calli, proliferation of calli, days to callus induction, callus morphology, accumulation of osmolytes like proline, concentration of cellular ions like Na^+ and K^+ , protein content etc., have been used as indices to measure salt tolerance. `Research on creation of mutant lines using radiations and analysis of somaclonal variants has also been carried out to derive salt tolerant rice lines.

Soil salinity causes increased uptake of Na^+ and Cl^- ions and decreased uptake of particularly K^+ and Ca^{2+}. If the uptake of Na^+ and Cl^- exceeds the plant's ability to partition the ions between different tissues or organs or to sequester the ions within the cells vacuole, these ions build up in the cytoplasm to toxic concentrations. Potassium is reported as a major osmoticum in plant cells under high salt concentrations [17]. Bal et al. (1986) reported that salt tolerant rice varieties accumulate lesser Na^+ and higher K^+ than susceptible varieties [18]. Lower Na^+:K^+ ratio has been found to be a characteristic of saline tolerance in rice. Elevated levels of Na^+ can induce deficiency of the essential element K^+ and induce deleterious changes in protein conformation [19].

Compatible solutes like proline, sucrose, polyols, trehalose and quaternary ammonium compounds (QACs) such as glycine betaine, alinine betaine, proline betaine and pipecolate betaine accumulate during stress conditions [20,21]. They provide protection to plants from stress by contributing to cellular osmotic adjustment, reactive oxygen species detoxification, protection of membrane integrity and enzymes/protein stabilization [22,23,24]. Proline, an amino acid, plays a highly beneficial role in plants exposed to various stress

conditions. It acts as a metal chelator, an antioxidative defense molecule and a signaling molecule [25]. Proline contributes to stabilizing sub-cellular structures scavenging free radicals and buffering cellular redox potential under stress conditions [22].

Calli morphology

Embryogenic calli derived from rice genotypes can be analysed for their morphology on salanised media. At the end of callus proliferation period (21 days), the morphology of the calli can be recorded and scored. For scoring, rating scales of 1-9 have been used [26]. Similar scoring method was followed by Sankar et al. (2009) as shown in Table 1[27]. A review on the calli screening for salt tolerance is given in Table 2.

Table 1: Calli morphology score

Score	Description of calli
1	Completely turns black to dark brown, dead calli
3	Watery / sticky appearance, more than 75 per cent of calli turns brown
5	Yellow to brown in colour, water soaked, slimy surfaced calli
7	Yellow to pale yellow, water soaked areas interspersed with pale yellow friable calli
9	Pale yellow to white in colour, healthy, nodular and friable calli

Table 2: Brief reports on calli screening

Reported by	Observation
Reddy and Vaidyanath (1985)	Callus growth decreased with increased NaCl concentration. Proline accumulation was enhanced several fold [28].
Pushpalatha (1994)	No callus growth was observed at a NaCl concentration of 2.5 % (w/v) [29].
Lutts et al. (1996)	Exposed, mature embryo derived calli of I kong Pao (salt sensitive), Aiwu (moderately resistant) and Nona Bokra (salt resistant) to three iso-osmotic concentrations of NaCl, KCl, Na_2SO_4, artificial sea water and mannitol. KCl was the most detrimental to callus growth. Na^+ and Cl^- accumulations as well as internal osmotic potential were lower in Nona Bokra and in Aiwu, suggesting a cellular component of salt resistance in these genotypes. Proline was seen as a symptom of injury in stressed rice calli [30].
Chauhan et al. (1997)	Ability of cells to maintain higher concentrations of K^+ and lower levels of Na^+ and Cl^- coupled with the maintenance of higher concentration of sterols and polyamines contributed to salt tolerance in rice callus [31].
Chauhan and Prathapasenan (1998)	Calluses of salt tolerant (Bhoora rata) and salt susceptible (GR 11) rice cultivars were cultured on Linsmaier and Skoog's medium containing LD_{50} concentrations of NaCl (200 mM) and hydroxyproline (10 mM). Resistant cell lines derived from both cultivars showed increased dry weight and proline content [32].
Thach and Pant (1999)	CSR27 (salt tolerant) and HBC19 (salt sensitive) varieties were assessed for *in vitro* salt tolerance. Fresh weight of calli decreased with increasing salt concentrations. Proline content was higher in the callus of CSR27 when compared to HBC19 and hence can be used as an indicator for salt tolerance [33].
Pushpam and Rangaswamy (2000)	There was a decline in callus growth and score at higher concentration of NaCl compared to low levels. Decline in growth of callus in the NaCl environment was due to diversion of some quantum of energy for growth and metabolism [26].

Narayanan (2000)	Studied callus induction of rice cultivars on MS medium supplemented with five different concentrations of NaCl (0.3 % to 1.5 %). He observed that IR 20, White Ponni, CSR 10, TRY 1 and CO 43 were salt tolerant [34].
Shanmuganathan (2001)	Three rice hybrids CORH 2, DRRH 1 and PSD 1 were identified as salt tolerant and hybrids KRRH 1 and CNRH 3 as moderately salt tolerant by *in vitro* screening [35].
Miki et al. (2001)	Based on *in vitro* studies on salt tolerant rice cv. Nipponbare they hypothesised that *in vitro* step up salt selection induced the capability to maintain no lethal concentration of NaCl in the leaves [36].
Basu et al. (2002)	Presence or retention of K^+ in rice callus was a key factor for salt tolerance as it was found to be positively correlated with growth. Proline was probably the last metabolic device that rice calluses opted for when exposed to salt stress [37].
Babu (2002)	Conducted *in vitro* screening experiments on 13 parents and 36 hybrids for salinity tolerance. TS 29, Pokkali, Vytilla 1, TRY 1, BTS 24, TS 29 x BTS 24, TS 6 x BTS 24, TS 6 x Vytilla 1, TS 6 x TRY 1 and IR 58025 A x Vytilla 1 were tolerant to high levels of NaCl concentrations [38].
Leelavathy (2002)	At higher concentration of NaCl, the saline tolerant parents Pokkali and CSR 10 registered maximum score for callus morphology. Accumulation of proline, protein, Na^+ and K^+ were found to be maximum at one per cent NaCl, while Na^+/K^+ ratio was minimum in tolerant cultivars. The rate of accumulation of proline and protein was much higher in tolerant cultivars [39].
Pushpam (2003)	Screened somaclones for salt tolerance in rice. Somoclonal variants in the R_4 generation, derived from IR 50 and IR 20 rice cultivars were subjected to various levels of NaCl to evaluate their tolerance to salinity. S-11, S-77, S-79, S-88b, S-97 and the awned variant S-58 were found to be the most salt tolerant somaclones [40].
Saleem et al. (2005)	Embryogenic calli of Basmati 370 cultured on MS medium containing 9.05 µM 2,4-D was subjected to irradiation at 50 Gy of gamma rays of ^{60}Co for creating genetic variability for salinity tolerance. NaCl adapted irradiated callus showed 2.0%-4.75% regeneration frequency on MS regeneration medium containing 5.37 M NAA and 9.29 µM Kinetin. Two putative lines (M_2 generation) with moderate salt tolerance were obtained at seedling stage [41].
Sankar et al. (2009)	Analysed five temperature sensitive genic male sterile lines (TGMS), eight salt tolerant testers and 40 hybrids obtained by crossing them in line x tester design for salt tolerance under *in vitro* condition. Hybrid GD 98029/CSSRI 13 ranked first followed by hybrids GD 98028/CO 43, GD 8028/CSR 23, GD 98029/CSR 10, GD 98029/Nona Bokra and the parent Nona Bokra in MS media supplemented with 2,4-D 2 mg l^{-1}, kinetin 0.25 g l^{-1} and casein hydrolysate 1 g l^{-1} along with NaCl at 1.6 per cent concentration exhibiting their potential for salt tolerance [27].
Shanthi et al. (2010)	Genotypes Pokkali, CSR 10, TRY 1, TRY2, White Ponni and BPT 5204 were screened for salt tolerance *in vitro* using embryogenic calli derived from them. Pokkali exhibited higher callus development and regeneration at Nacl concentration of 150mM followed by TRY 2 and CSR 10 [42].
Priya et al. (2011)	A 6.5 fold increase in proline content in **callus** culture of indica rice cv. IR 64 was

	observed suggesting proline accumulation as an index of salinity [43].
Zinnah et al. (2013)	Studied BRRI Dhan 38 and Chini Kanai to obtain salt tolerant lines through somaclonal variation. Plant regeneration in BRRI Dhan 38 was observed till 100 mM NaCl concentration and in Chini Kanai till 150 mM NaCl concentration [44].
Hasan and Sarker (2013)	Jirabhog and Badshahbhog showed better callus formation under salt stress among eight aromatic rice varieties [45].
Rudra et al. (2013)	Mature seed scutellum was cultured on MS-based medium supplemented with different concentrations of NaCl from 0.2% to 1.5%. Cultivars Rajashail and Katicota were found to be best responsive [46].
Zahid et al. (2014)	*In vitro* screening for salt tolerance in aromatic rice genotypes were carried out. Shakkhorkhora exhibited tolerance and regeneration was seen till 0.6% NaCl concentration while Basmati was least tolerant [47].
Attia et al. (2014)	Egyptian rice cultivars were induced on LS medium supplemented with cobalt sulfate (5 mg/l). Cobalt sulfate decreased the negative impact of salt stress [48].
Siddique et al. (2014)	Parameters like viability of **calli**, relative growth rate, tolerance index and relative water content were used as indices to measure tolerance in the rice genotypes BR10, BRRI dhan32 and BRRI dhan47. BRRI dhan47 performed well in MS media supplemented with 2, 4-D (2.5 mgL^{-1}), Kin (1.0 mgL^{-1}) and 11.7 gL^{-1} NaCl. Desiccated **calli** showed better capability to survive in NaCl induced abiotic **stress and gave** 1.9 fold increased regeneration in 11.7 gL^{-1} **salt** level for BRRI dhan47 [49].

CONCLUSION

There is an essential need for improving stress tolerance is majorly consumed food crops like rice. Yield improvement to feed the ever growing human population alone will not solve the problem as losses due to biotic and abiotic stresses will bring down the yield of a crop. Introgressing genes responsible for stress tolerance can only give us a long term solution due to building up of horizontal resistance. *In vitro* screening can serve as an efficient tool in identification of stress tolerant genotypes and traits involved in tolerance.

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