Tissue Culture Technician and in Vitro Screening of Rice (Oryza sativa L) Callus for Salt Tolerance

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Abstract

World wide, the most important cereal crop is rice (Oryza sativa L.) However, its production goes through from salty environments in several areas since it is one of the salt perceptive crops. NaCl mainly contributes salinity that is one of the significant abiotic stress aspects and, the leading cause of crop failure in the world. Tissue and cell culture methods have been applied to gain salt tolerant plants through using in vitro culture methods. In the present paper, the vitro screening of plant germplasm for salt tolerance, as a successful employment of this technique in rice is raised. The current review, focus on plant tissue culture as a method applied for in vitro to protocol for callus induction and screening of salt tolerance and revival of rice in the laboratory.

Keywords: Tissue culture, callus induction, regeneration, NaCl, salt screening.

Introduction

The most important food crop is rice (Oryza sativa L.) in the world and supplies over half of the worldwide population [1]. Rice plays a significant role as a staple food crop and is applied to feed more than 3 billion inhabitants on a daily calorie intake of 50 to 80% [2,3]. In agriculture, global, salt stress is a leading abiotic stress, with an approximated 20% of the Earth’s land collection and practically half of all irrigated land influenced by salinity.

Improved salinization of arable land is anticipated to have distressing global results, with calculations of 30% land loss within the next 25 years, and up to 50% by the year 2050[4,5]. Rice efficiency in numerous parts of the world is consequently severely limited by salinity on the explanation of the occurrence of irrigation in rice farming. In plants, tolerance to salt toxicity is a physiological and genetic multifaceted trait. Salt tolerant plants (Halophytes) are diverse from the salt sensitive (Glycophytes) in terms of the peculiarities in their structure, facility to requisition otherwise toxic ions, and other physiologic procedures [6].

So, the numerous studies to enhance salt tolerance of crop plants an importance to increase the productivity of agriculture [7]. The tissue culture method has appeared as a cost-effective and feasible substitute instrument for expanding stress tolerant plants in recent years. The efficiency of many commercial crops is limited by primary abiotic stresses counting mineral toxicities, waterlogging, drought, frost, heat, and salinity [8]. In tissue culture method using plant cells or tissues are useful instruments for salinity studies, which ease to loosen the salt tolerance mechanism and halophyte plants, at the organized tissue or unorganized cellular level, and may present information on the probable for biochemical, physiological, and development reactions to salt stress at diverse levels of tissue organization.

Also, in vitro studies consent to comparatively faster reactions, controlled environment and shorter generation time as evaluated to ex vitro situations [9]. Tissue culture of rice may facilitate to get somaclone, and their concert can be monitored in the field. Physical and chemical mutagenic instruments treated organs create altered callus that as well assist to gain disease, semicolons, insect or pest resistance, stress or salt tolerant distorted line of rice.

The somaclonal difference could be controlled by shifting explants, medium, particularly the phytohormones in medium,
culture techniques and duration of time spent in vitro [10]. The current review is an original contribution to the wide-ranging depository of studies in tissue culture of rice (Oryza sativa L.) and screening of callus for salt tolerance.

**Tissue Culture Studies in Rice**

The growth of a high proficiency in vitro plant renewal system is required for the development of the crop by several modern biotechnological means, for example, breeding by genetic transformation and somaclonal difference [11]. Li-na et al. [12] also reported that the tissue culture of plants from grown-up seed of rice has numerous benefits such as the supply of plant fabric without limitation of geographical and season environment, and with a simple procedure and less infectivity by microorganisms. Consequently, this equipment is applied extensively by numerous specialists of rice biotechnology. It has been stated that plant tissue culture methods are functional to through breeders to produce new ranges of crops.

*In vitro* culture of the plant have been normally applied by plant breeders for many decades and has resulted in several varieties that are edible and grown as food all over the world, as well varieties have developed well in a planters’ field, and have not reasoned any health troubles. The amount of genetic difference as an outcome of tissue culturing is mild and manageable; more alterations are an essential part of plant breeding [13]. The function of biotechnology in arrangement with common breeding techniques may assist to enhance food production appropriately. Proficient plant renewal through *in vitro* micro propagation is very critical for the successful exploitation of biotechnology in rice crop development [14].

**Micro Propagation**

The technique of micro propagation is typically divided into different phases, i.e., prepropagation, beginning from explants cell or tissue, subculture from explants for propagation, shooting, as well rooting and hardening. These steps are internationally appropriate for large-scale development. The delivery of adjustment small micro-propagated of plants to farmers and marketplaces, also, needs careful management [15]. In rice *in vitro* cultures, uncovered seeds were applied most generally as explants for inducing callus for the reason that they are obtainable year round, simple to sterilize and have usually higher renewal probable of the callus than that from other sources such as, shoots, leaves and also roots [11]. Tariq et al. [16] also observed callus cultures are particularly significant in plant biotechnology. Management of concentration between cytokinin to auxin proportion in the average can guide to the expansion of roots, shoots, or somatic embryos from which whole plants can consequently be created. Callus culture can furthermore be applied to, begin cell postponements, which are applied in a diversity of ways in plant revolution studies.

**Callus Induction and Proliferation in Rice**

Improvement towards the transfer of functional genes into indica rice has been slow. Numerous features have been observed to progress the regularity of produce callus and plant renewal in rice. The superiority of the calli of rice is one of the leading features to conclude the rate of renewal. It has been stated that one of the leading features to progress the quality of the calli is the composition of the plant tissue culture media [17]. Winicov [18] found that the callus of rice induce in the dark, on LS media complemented with different concentration 1 mg/L 2,4-D, of 0.3 mg/L kinetin and 100 mg/L tryptophan with agar indica rice diversities IR 28, IR 42 and Pokkali or MS media complemented with different concentration of 2 mg/L 2,4-D, 0.2 mg/L kinetin, and 100 mg/L tryptophan with agar, from the seed of two creams of the crop US rice lines (M-202 and L-202). Moon et al. [19] studied, embryogenic rice calli were stimulated from grown-up rice varieties, Sasanishiki seeded on solid N6 media complemented with 12 mM proline, 0.1 g/L casamino acids, 5mM 2-(N-morpholino) ethanesulfonic acid monohydrat (MES), 4 mg/L 2,4-D, 10/L sucrose, 30 g/L sorbitoland 0.2% Gelrite. Tsugawa and Suzuki [20] have also reported, the embryogenic callus of rice Nipponbare was stimulated from seeds by culturing them for 1 month on (MS) medium enclosing concentration of 2 mg/L 2,4- D and 0.5% Gelrite. Lee et al. [21] investigated the callus induction for three cultivars, japonica rice Nak-Dong, Hwa-Chung, Dong-Jin, were applied to the embryogenic callus induction.
from mature seeds. Callus production in three media, N6, MS, and LS, were examined for the instruction of the whole embryogenic calli and non-embryogenic plus embryogenic. All the media stated above under shadow were complemented with concentrations of 2,4-D 3.0 mg/L, casein hydrolysate 1.0 g/L. The appearance time, colour, shape, size, and number of the induced embryogenic calli, differed among the rice cultivars depending on the kind of basal medium (LS, MS, and N6). The presence of sufficient amount of sucrose in the medium was a complete prerequisite for embryogenic callus configuration and shoot induction. Introduction of the embryogenic calli, whose general rates varied from 30 to 56%, was most proficient in N6 medium complemented with 2, 4-D 3.0 mg/L and sucrose 30 g/L.

Ullah et al.[22] during their work studied the callus induction of two rice varieties, Basmati-385 and Basmati-370, applying N6 media and MS media complemented with (2,4-D 2.0, BAP 0 mg/L), (4-D 2.0, BAP 0.1 mg/L) and (2,4-D 2.5, BAP 0.5 mg/L). However N6 media were confirmed to be best and best reactions toward callus instruction was monitored for variety Bas-385 on both N6 and MS media. Calli achieved from Bas-385 were vigorous and friable as compared to Bas-370. Variety Bas-370 react reasonably on the N6 and MS media at different arrangements of BAP and 2,4-D. Bas-370 on MS media complemented with BAP 2.0 mg/L, and 2,4-D at 0.1 mg/L provided good concert towards callus instruction. In general, results signified that best callus was stimulated on the N6 and MS when complemented with 2,4-D 2.0 and BAP 0.0 mg/L for variety Bas-385.

Tariq et al.[16], studied four varieties of rice Fakhre Malakand, Basmati-371 Super Basmati and Basmati-370, to expand an efficient procedure for optimal to produce callus. Using in this experimenter N6 and MS media, including hormone 2,4-D in different concentrations (2.0, 2.5 and 3.0 mg/L), 0.6% agar (Difco), 3% sucrose, at 16-8h light/dark, were applied for callus instruction. Fakhre Malak and created the highest callus on N6 media, including 2,4-D 3 mg/L although other three diversities demonstrated highest callus instruction in N6 media including 2,4-D 2.5. Mg/l for callus induction, N6 was discovered well than MS media. In vitro culture technique of mature seeds of rice indica and japonica from three varieties LX278, PSB Rc58, and PSB Rc28, to create callus apply, 30 ml RS medium complemented with 2,4-D 2 mg/L and NAA 1 mg/L and hardened with phytagel 2 g/L and Gelrite agar 2 g/L, in the dark at 25°C until callus showed, demonstrate the seeds from three varieties had differing reactions to callus induction[23]. Zhao et al.[11] observed the response of callus induction to four indica diversities, Xiyezhan, Jingxian 93, Qimiaoxiang and Qiuguiai 11, and one japonica variety, Taipei 309, The medium applied of N6 organic elements, N6 macro elements, 2,4-D 2 mg/L, proline 500 mg/L, and sucrose 3%.

It was shown that the explants at lower development had lower rates of callus introduction and lower construction of callus masses than those with the more developed ones in the innovative cultures. While the superiority of the callus stimulated from the younger explants was much better since the proportion of somatic embryogenic to non-somatic embryogenic callus created by younger explants was superior and raised quicker in the following production cultures. With regard to fresh immature seed-explants and dry ones at the same development, dry ones commonly reacted better regarding the ratio of callus quality and the introduction of callus. Li-nan et al [12] investigated the mature embryos to callus introduction, diverse rice varieties, eleven hybrid rice, nine indica and nine japonica ranges, to induce callus for all the rice varieties, apply M8 media sand complemented with, 2,4-D 2 mg/L, sucrose 30 g/L and agar 8 g/L.

The diverse applications of NAA, ABA, kinetin, 6-BA, and sorbitol for callus introduction. For training of diverse media, the complemented MS media was applied as for the diverse media for rice diversities in the present study. For japonica, MS basal medium was supplemented with 6-BA 1.0 mg/L, sucrose 30 g/L, agar 8 g/L, 2,4-D 0.5 mg/L, and activated carbon 1 g/L; for hybrid and indica rice, the MS basal medium was complemented with sucrose 6-BA 0.5 mg/L, agar 8 g/L, 30 g/L activated carbon 1 g/L, and 2,4-D 0.2 mg/L. The superior callus induction percent was observed when 6-BA 0.3 mg/L, 6-BA 2 mg/L and kinetin 0.5 mg/L and NAA 1 mg/L were added in the M8 media, supplemented with sucrose 30 g/L, agar 8 g/L.
and 2,4-D 2 mg/L, for japonica, indica and hybrid rice varieties, respectively. Researching of aromatic rice on, two varieties, BRRI Dhan 34 (Khakhani), and BRRI Dhan 50 (Bangla Moti), used for callus induction from dehusked mature seeds, were applied to set up an appropriate system for callus commencement. Applying MS medium, complemented with diverse attentions, i.e. (1.0, 2.0, 3.0, 4.0 mg/L of 2,4-D and combination with BAP 2.0 mg/L), with 0.7% agar. MS media complemented with only 3.0 mg/L/2,4-D, created the highest percentage of callus that is (80%) for BRRI Dhan 34 and (90%) for BRRI Dhan 50. Alternatively, MS medium with 4.0 and 3.0 mg/Lof 2,4-D in arrangement with 2.0 mg/LBAP created a maximum percentage of callus (70%) for BRRI Dhan 34 and (80%) for BRRI Dhan 50 [10].Damanik et al. [24].it was found that, Malaysian rice mutants, MR219-9 and MR219-4, and FR13A cultivar, callus introduction.

The mature seeds were used for culture on MS media supplemented with 2.4 D 2.21 mg/L, gelrite 2.75 g/L, casein hydrolysate 0.4 g/L and sucrose 30 g/L. The cultures were protected under the dark circumstance. Zinnah et al.[25]also found that callus production for tow rice verities, CHINI KANAI and BRRI 38, mature seeds were used as explants. The basal medium MS was applied for callus introduction, with the attentiveness of expansion 2,4-D (0, 1, 2, 3,4,5 and 6 mg/L). It was observed that MS medium complemented with (5.0 mg/L, 4-D) and (3.0 mg/L,2,4-D) created a maximum percentage of callus, 75% for BRRI Dhan 38 and Chini Kanai, in that order.

Plant Regeneration in Vitro Rice

For the callus rice regeneration, cultivar Sasamishiki, applying MS media supplemented with, casamino acids 2 g/L, sorbitol 30 g/L, MES 5 mM, kinetin 1 mg/L, NAA 2 mg/L, sucrose 30 mg/L and Gelrite 0.4%[19]. In a study by Lee et al. [21] plant regeneration from callus to, three japonica rice cultivars, Nak-Dong, Dong-Jin and Hwa-Chung. The embryogenic callus was relocated on to two; MS media complemented with kinetin 1.0 mg/L, and NAA0.1 mg/L was tested with diverse attentiveness of sucrose(0, 15, 30 and 50 g/L) and agar (Sigma) as a gelling agent 1.0 or 1.6% (w/v). The other including 1.6% agar and sucrose 30 mg/L was tested with diverse attentiveness of NAA (0.1, 0.5, 1.0 and 2.0 mg/L), and kinetin or BA (0.5, 1.0, 2.0 and 4.0 mg/L) under the permanent light. Kinetin was set up to be more effectual for shoot renewal compared with BA, while the maximum shoot renewal frequencies were monitored when either cytokinin was joined with high attentiveness NAA 2.0 mg/L. The best attentiveness of kinetin for the maximum shoot renewal occurrence (67−77%) was diverse among the cultivars tested.

Proficient plant regeneration system in vitro from 3, 5, 7 and 9-days-mature root sections of four indica rice genotypes, Moulata, BR22, BRRI Dhan 29 and BR5842-15-4-8, were. Genotypic results were practical in callus subsequent and induction plant renewal. Furthermore, the phase of expansion of the root explants as well played an essential role in callus subsequent and formation plant renewal. Younger explants were more proficient in both plant regeneration and callus induction. Plants renewed in vitro were productively instituted in soil and created productive seeds [26].Tariq et al.[16], studied mature seed rice two varieties, Basmati-371 and Basmati-370, callus induction and total plant regeneration for total plant renewal the callus of two diversities were placed on N6 medium including diverse attentiveness of NAA:BAP (1:2, 1:2.5 and 1:3 mg/L).

The highest renewal frequency (%) was monitored on N6 media including BAP 2.5 mg/Land NAA 1 mg/L. Embryogenic calli rice from three genotypes, LX278, PSB Rc58 and PSB Rc28, after two subcultures, were relocated to LSrenewal medium, LS inorganic salts, including, 100 mg/L inositol, 10 mL l−1 thiamine-HCl 30 g/L sucrose and supplemented with 1g/LMES buffer, 30 g/L sorbitol, 2 g/L casein hydrolysate, 1 mg/L NAA, 2 mg/L BA, and with 4 g/Lphytagel, to solidified. Calli were exposed to 8 h of dark and 16 h of light period daily. Every month, calli were relocated in the new medium until regenerates emerged.

To regenerated shoots were stimulated to root in MS media complemented with picolinic acid 1 mg/L. Outcomes demonstrated that while the three genotypes were all able of shaping calli, PSB Rc58 and PSB Rc28 were not capable of maintaining development and commonly distinguished roots 55.3% or became necrotic 76.9%, in that
order. LX278 was the most agreeable to tissue culture, shaping 78.8% of embryogenic calli. When half of this was transported in renewal medium, 69.8% renewed saplings. Among the plantlets, 48.9% expanded by somatic embryogenesis and 51.1% by organogenesis. Li-na et al. has also reported, plant regeneration medium for callus, rice diversities, nine indica, nine japonica and eleven hybrid rice diversities applying MS basal medium complemented with PPP 0.4 mg/L, NAA 0.2 mg/L, sucrose 15 g/L, Landagar 8 g/L, was applied as a renewal medium for all the three rice diversities.

The renewal rates were for indica 3.6-87.5%, japonica 9.2-59.5%, and hybrid rice. Plantlet regeneration for callus two diversities of BRRI Dhan 34 (Khashkhani), aromatic rice, and BRRI Dhan 50 (Bangla Moti) apply MS medium with BA 2.0 mg/L, NAA 1.0 mg/L and different applications of Kinetin (0.0, 1.0, 2.0, 3.0, 4.0 mg/L) were used, 8 hours dark /16 hours light. The highest percentage of shoot renewal was proved at MS medium complemented with 2.0 mg/L BA, 1.0 mg/L, NAA and 4.0mg/L of Kinetin, for both diversities.

Salt Tolerance Studies in Vitro Rice Plants

In recent years, significant improvement has been made concerning the expansion and separation of pressure principally salt tolerant callus/cell lines applying in vitro technique. In vitro choice will save the time needed for expanding abiotic stress tolerant lines of significant plant variety. Binh et al., studied cells of rice, excremental, developed under 1.5% NaCl stress for 3 months, provided an increase to plants throughout embryogenesis in diverse salty situations. The high renewal probable 59.6% of salt-free medium reduced quickly with rising attentiveness of salt in the renewal medium.

At 1.25% NaCl, healthy shoots were expanded in 14.9% of the cultures. Under 1.5% salt stress, embryo configuration and embryo germination 6.1% was monitored but further expansion into plants was introverted. Cells not pretreated with salt created plants at a low occurrence 2.6-4.2% both in low saline and salt-free situation. 0.75-1% NaCl. Cells pretreated for 3 months with 0.75% salt did not present increase to plants on all experienced media. Babu et al. have also reported, tissue culture four male disinfected line rice verities, TS 29, TS 6, IR 58025A and COMS 9A were applied as lines and nine varieties recognized for salt tolerance specifically CSR 27, CSR 13, Pokkali, Vytilla 1, CO 43, TRY 1, Jaya, Vytilla 2 and BTS 24. To create callus mature seedin (MS medium and 2,4-D 2 mg/L), (MS medium, 2,4-D 2 mg/L and Casein hydrolysate 1 g/L), (MS medium, 2,4-D 3 mg/L and Casein hydrolysate 1 g/L) than callus were relocated to salt stress medium including (MS medium, 2,4-D mg/L and kinetin 0.25 mg/L) along with diverse five attentiveness of NaCl salt (0.2, 0.4, 0.6, 0.8 and 1.0%) with the intention of screen the salt tolerant, the outcome demonstrate callus development reduced with rising NaCl attentiveness in the medium. TS6/ Vytilla 1, TS6/BTS 24, TS 6/TRY 1, IR 58025 A/Vytilla 1 TS 29/BTS 24 and were the hybrids with high tolerance to salt stress in vitro. Ahmad et al. during their work studied the, dehusked seeds of two indica rice genotypes, Basmati-Kashmirof and Basmati-370 applied for the commencement and continuation of callus was LS medium, complemented with 0.5 mg kinetin and 2.5 mg/L 2,4-D One-month-old calli cultured in liquid LS media complemented with the same attentiveness of 2,4-D and kinetin as applied for callus initiation and iso-osmotic managements expanded by polyethylene glycol (PEG-8000) and NaCl. The outcome demonstrates, the degree of stress tolerance of both genotypes was better for PEG stimulated stress than for NaCl stimulated stress. The comparative growth rate of callus was decreased under both stresses; though, the overturn was true for callus dry weight. Sodium Na+ substance of the callus tissue was enhanced just under NaCl stimulated stress. Salt stimulated stress decreased Ca2+ and K+ substances, although the PEG stimulated stress enhanced them. Superior levels of stress enhanced the proline substance several collapses with more enhance being under PEG stress than that under NaCl. Osmotic and water potentials of the callus tissue reduced, while turgor potential enhanced under both abiotic stresses. In general, Basmati-370 was more tolerant to both PEG and NaCl stimulated stresses than Basmati-Kashmir, due to less decrease in development and more dry weight.
Furthermore, Basmati-370 gathered higher amounts of cations, free proline, and preserved maximum turgor as compared to Basmati-Kashmir. At the cellular level, mechanism of Na Cl stimulated osmotic stress tolerance was set up to be connected with the more ionic growth of inorganic solutes and that of PEG stimulated osmotic stress tolerance with the gathering of free proline. Outcomes of salt stress on some biochemical and physiological features were examined in three rice cultivars varying in salt-tolerance capability two cultivars, Pokkali, KDML105 and Leuang Anan. In an experiment using dehusked seeds were germinated on MS medium, 6% agar solidified, for 7 days, and after that, the nearly-size seedlings were chosen and relocated to culture on MS medium inserted Na Cl at attentiveness of 0, 50, 100, 150 and 200 mm for 15 days.

The outcomes demonstrated that all three cultivars of rice seedlings developed under high salinity had shoot and root length, dry and fresh weight of shoot, and qualified development rate of shoot reduced, while the Na+/K+and proline ratio substance of leaf were enhanced. Pokkali accrued the lowest amount of proline while KDML 105 was the maximum. Additionally, Pokkali demonstrated the lowest K+/Na+ proportion while Leuang Anan was the maximum [30],Shanthi et al.[31]observed the effect of, in vitro screening method with diverse attentiveness of NaCl stress for seven genotypes consist of salt tolerant (TRY 1, TRY2 and Pokkali, CSR 10), moderately tolerant to salt (BPT 5204 and White Ponni) and sensitive (IR 29) were applied embryo culture method. Callus was commenced in MS media, Kinetin 0.5 mg/Land 2,4D 2 mg/L with sucrose (3%) 30 grams and agar (0.8%) 8 grams and diverse attentiveness of different concentration of Na Cl (50, 100 and 150 mm) were inserted with the media to generate salt stress. Result show disclosed that all the genotypes and management and their communication result were considerably diverse from each other.

Among the seven genotypes studies, Pokkali was considerably higher for callus introduction with 62 % pursued by CSR 10 (58 %), TRY(R) 2 (54.5 %) and TRY1 (53 %) with, and in that order. Pokkali scheduled the maximum level of callus improvement (35%) even at high level of Na Cl stress (150mM) pursued by CSR 10 (24%) and TRY 2 (25%). When the callus for these genotypes was transported to renewal media in the same level of Na Cl stress (50, 100 and 150mM) maximum level of regeneration of shoot was appreciated in Pokkali (37.5 %) pursued by TRY (R) 2 (25 %) and CSR 10 (31 %).From these examinations, it was concluded that somaclones achieved to form the diversities of Pokkali, TRY(R) 2 and CSR 10 could be assessed additional in the natural ground circumstance to expand a high deferring salt tolerant diversities or could be applied as a contributor for the enhancement of salt tolerant for varieties. Zinnah et al.[25] remained four-weekold callus rice varieties, CHINI KANAI and BRRI 38 relocated against the same medium those were applied for plant renewal, complemented with diverse NaCl attentiveness, for instance, (0, 50, 100, 150 and 200 mM ), for salt stress reactions. Callus on managing medium NaCl (0 mM) was showed common propagation. With the growth in NaCl attentiveness, there was a slow reduce in callus fresh weigh. Plant regeneration of BRRI than 38 was 80% at NaCl (0 mM) but reduced to 20% at NaCl (100 mM). There was 0% plant renewal at NaCl (150 mM) for BRRI 38 and Chini Kanai in that order. In Chini Kanai plant renewal on the no-stress medium was 60%. At 150 mM it reduced to 20%, and there was no renewal at NaCl (200 mm). It shows that Chini Kanai is more salt tolerant then BRRI Dhan 38.

**Conclusion**

There is a worldwide apprehension on raising the cultivable area under salinity. Rice is the main crop used by most of the human inhabitants around the world, and its command is constantly enhancing. Developing the surrender of rice under salt had an effect on areas is an instantaneous prerequisite. The services provided by breeders towards solving the trouble are substantial [32]. Successful function of biotechnology to the salinity restrictions facing crop plants will need both a good biological knowledge of the aim species and the instruments fundamental tolerance to this stress [33]. In vitro tissue culture could be a significant means of humanizing crop tolerance and surrender throughout genetic alteration as well as by stimulated somaclonal deviation. Consequently, it is
significant to develop a proficient procedure of callus pro-life allocation to create in vitro variety for salt stress tolerance, and to expand chances for genetic management of rice throughout tissue culture, counting attempting different media and explants [25]. Considering the differentiations engaged in NaCl confrontation at the plant and callus level, it is not shocking why all plant lines renewed from NaCl-chosen callus rows were not NaCl-resistant. The NaCl-resistant plant rows expanded here to hold the possible to clarify the molecular instrument engaged in rice NaCl resistance in addition to in rice breeding for NaCl resistance [34]. Among the diverse tissue culture methods the one of the significant methods is grown-up embryo culture in rice to generate extra difference and original rice diversities [35]. Therefore, the salt tolerance can be considered as a feature of embryogenic callus of rice under in vitro culture situations. This in vitro method with diverse NaCl stress can as well be applied as a screening method for salt tolerance rather than the field screening, as field screening would take more time [31].

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