

# Identification of Candidate Genes from the Proline-polyamine Pathway to Look for Desirable Alleles for Development of Salt-tolerant Rice

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## Abstract :

Rice is a glycophyte plant. It cannot withstand excess salt in the soil. Since soil salinity is increasing at an alarming rate due to both natural and anthropogenic causes, it is a challenge for the plant scientists to find out new means to support growth and production of rice in salt-prone lands. This review article focuses on the detrimental effects of salinity on rice plants and ways to combat it. Of the numerous reported physiological, biochemical and molecular responses of the rice plants, the present review emphasise on the role of proline, polyamines and the inter-dependence of proline and polyamine to alleviate salt stress in rice. It also explores the possibilities of developing salt-tolerant rice with specific reference to the proline-polyamine pathway. The approach is looking for candidate genes of the critical enzymes in the proline-polyamine pathway and exploration of different allelic forms of the candidate genes in hitherto unexplored and less cultivated rice landraces, which have the inherent capacity to tolerate salt stress.

**Keywords :** Salt-tolerant rice, proline-polyamine pathway; candidate genes

## Introduction

Salts are present in the soil in their ionic forms. A particular soil is a salt-stressed one when the concentrations of cations ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ , and  $\text{Ca}^{2+}$ ) and anions ( $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{HCO}_3^-$ ) are high. These ions result from more water-soluble salts such as  $\text{NaSO}_4$ ,  $\text{NaHCO}_3$ ,  $\text{NaCl}$  and  $\text{MgCl}_2$  or less water-soluble salts like  $\text{CaSO}_4$ ,  $\text{MgSO}_4$ , and  $\text{CaCO}_3$ . The cause behind salinity in soil may be natural (e.g. mineral erosion in soil) or anthropogenic (irrigation, application of fertilizers). It is one of the significant abiotic stresses and affects the productivity of many crops all over the world<sup>1</sup>. High salt concentration in the soil affects the rice plants in all stages of their life cycle starting from the seedling to the flowering stage. At some points, the effects are more pronounced whereas rice plants can combat some effects. For example, during germination, active tillering and towards maturity rice plants are relatively salt-tolerant, but they are highly sensitive during the early reproductive and seedling stage<sup>2,3</sup>.

## Effects on root and shoot growth

The first effect of salinity stress in rice is a rapid decrease in growth, and stomatal conductance<sup>4,5</sup>. Height of the rice plants is severely affected due to salt stress at the vegetative, reproductive and seed ripening stages. Shoot growth is more affected than root growth due to salinity<sup>6</sup>. A rapid decline in root growth also takes place due to  $\text{NaCl}$  stress<sup>7</sup>.

## Effects on leaf growth and development

Leaf growth is severely affected due to salinity stress<sup>8,9,10</sup>. The reason behind this might be that uptake of the high amount of salt increases the toxicity level in adult leaves. Consequently, those leaves undergo early senescence thus decreasing the photosynthetic leaf area of rice<sup>11</sup>. Salt imparts three more stresses which are osmotic, ionic and oxidative stress<sup>12</sup>. Rice plants face osmotic stress during early periods of salt stress and as a result leaf development is impaired whereas as a result of prolonged salinity stress rice plants face ionic stress, and there is early senescence of older leaves<sup>13</sup>.

## Effects on reproductive stage

Salinity causes delayed heading in rice and adversely affects many of the yield components<sup>14</sup>. The yield components include panicle length, spikelet number per panicle and grain yield<sup>15</sup>. High salinity can cause a substantial reduction in the filled grains in rice plants which suggests that high salinity causes grain sterility in rice. Its productivity is severely affected by the presence of a higher concentration of salts in the soil<sup>16</sup>. Due to salinity pollen availability in rice is decreased and as a result seed set and yield also reduces. It also delays panicle emergence and flowering<sup>17</sup>.

In a nutshell, as a result of rice plant's increased sensitivity to salinity in the reproductive stage, there is delayed flowering, a decrease in the number of productive tillers and fertile florets per panicle and a reduction in individual grain weight<sup>17, 18</sup>.

### Physiological, biochemical and molecular responses of rice plants to salinity

Increased ABA synthesis is an early physiological response in rice plants. It is correlated with increased leaf and soil water potential due to osmotic stress in addition to decreased growth rate and lowered stomatal conductance<sup>19</sup>. From here it can be inferred that reduced stomatal conductance which is an early effect of salinity stress in rice plants may be due to the increased synthesis of ABA. Salt-tolerant rice varieties have a low level of constitutive and stress-induced ABA level in their leaves.

The most deleterious effect of salinity is the accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions in the plant tissue and soil<sup>20</sup>. Na<sup>+</sup> ions enter into roots through apoplastic and symplastic ways. Plants have their ways to inhibit this entry like the influx of salt into root itself prevents the entry of further Na<sup>+</sup> by activating specific signalling and perception mechanism in plants. It also reduces long-distance transport of Na<sup>+</sup> from root to shoot and restores leaf ion homeostasis.

These are the two short-term responses. Apart from these, long-term responses of plants to salinity are also present. Enhanced level of Na<sup>+</sup> in soil causes membrane damage. It also damages plant cell organelles and interferes with various plant physiological processes thus leading to cell death<sup>21</sup>. To be more precise, the effects of excess Na<sup>+</sup> concentration in soil includes increased ROS production and inability to quench them which leads to protein denaturation including protein of PS2, reduced photosynthetic rate and disruption of cellular structures.

To cope with these malfunction plants synthesize certain compounds known as osmolytes or compatible solutes which are harmless even at high concentrations within the cell. These osmolytes include sugars such as glucose, fructose, sucrose; complex sugars such as trehalose, raffinose, fructans; sugar alcohols like mannitol and glycerol; the amino acid like proline and amino acid derivatives like glycine-betaine, proline-betaine<sup>22,23</sup>. Proline plays multiple roles in alleviating the ill-effects of salt stress in plants. It scavenges free radicals, reduces cytoplasmic acidosis. It mainly accumulates in the cytoplasm where it functions as a molecular chaperone and stabilizes the distorted proteins. Some authors have related the accumulation of proline in the cytoplasm as a signal to the plant for salt and drought stress perception. The rate of proline accumulation increases under salt stress especially in the salt-tolerant rice genotypes, confirming its protective role against hyperosmotic stress<sup>24</sup>.

Apart from proline, many authors have also found that with increased salt stress there is an accumulation of polyamines in plant tissues<sup>25, 26, 27</sup>. Polyamines are polycationic and as a result of which they possess free radical scavenging properties, and therefore polyamines have antioxidant properties<sup>28</sup>. Roychoudhury et al.<sup>29</sup> found that the level of the diamine Putrescine (Put) was less in the salt-tolerant rice cultivar Nonabokra than the salt-sensitive cultivar M-1-48. On the other hand, the concentration of the polyamines such as spermine (Spm) and spermidine (Spd) increased in the salt-tolerant varieties, plausibly to protect the plant membranes from the harmful oxidized species. In Pokkali and Nonabokra, the two salt tolerant cultivars it was seen that the level of polyamines increased with increased duration of NaCl treatment<sup>30</sup>. However, it was also observed that the level of the polyamine Putrescine increased in the roots of Pokkali after short-term salt stress in contrast to a salt-sensitive rice cultivar IKP<sup>31</sup>.

### Pathways of Proline synthesis

Plants follow two pathways for proline biosynthesis - glutamate and ornithine pathway. The glutamate pathway is the major pathway for proline biosynthesis during osmotic stress. The precursor of proline in this pathway is glutamic acid. The enzyme  $\Delta^1$ -pyrroline-5-carboxylate synthetase (P5CS) catalyzes the formation of  $\Delta^1$ -pyrroline-5-carboxylate (P5C)<sup>32</sup>, an intermediate in the pathway which is then acted upon by the enzyme  $\Delta^1$ -pyrroline-5-carboxylate reductase (P5CR) to produce proline<sup>33,34</sup> (Figure 1). Proline is broken down in the mitochondria. First, the enzyme proline dehydrogenase or proline oxidase (PDH or POX) breaks down proline into P5C, and then the enzyme P5C dehydrogenase (P5CDH) converts P5C to glutamate<sup>35</sup>. In some plants, proline is synthesised from ornithine during seedling development and in some plants at the time of stress. In this pathway, proline is produced from ornithine by

the action of the enzyme ornithine- $\alpha$ -aminotransferase and is then transaminated to P5C<sup>36</sup> (Figure 1).

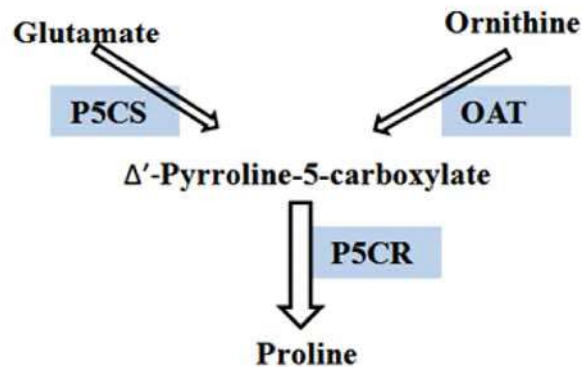


Figure 1

### Polyamine types and pathways of biosynthesis

There are principally three kinds of polyamines - putrescine, spermine and spermidine. These are present in all plant species. The polyamine biosynthesis in plants is a two-step process where the first step is the formation of putrescine from arginine and in the second stage spermine and spermidine are produced from putrescine. In the first step the enzyme arginine decarboxylase (ADC – E.C.4.1.1.19) decarboxylates the L-arginine to agmatine. It is followed by hydrolysis and deamination of agmatine to N-carbamoylputrescine by the enzyme agmatine iminohydrolase (AIH – E.C. 3.5.3.12). N-carbamoylputrescine is then hydrolysed, decarboxylated and deaminated by N-carbamoylputrescine amidohydrolase (CPA – E.C. 3.5.1.53) to form putrescine (Figure 2). Putrescine can also be synthesised by the direct decarboxylation of L-ornithine, catalyzed by ornithine decarboxylase (ODC – E.C.4.1.1.17). The polyamine spermidine is synthesised by the addition of an aminopropyl group from S-adenosylmethionine to putrescine by the enzyme spermidine synthase (SPDS – E.C.2.5.1.16), and spermine is synthesised by the addition of an aminopropyl group from S-adenosylmethionine to spermidine by the enzyme spermine synthase (SPMS – E.C.2.1.5.22) (Figure 2). S-adenosylmethionine is a common precursor for polyamines and ethylene biosynthetic pathways, and it is decarboxylated by the enzyme S-adenosylmethionine decarboxylase (SAMDC – E.C.4.1.1.50)<sup>37</sup>.

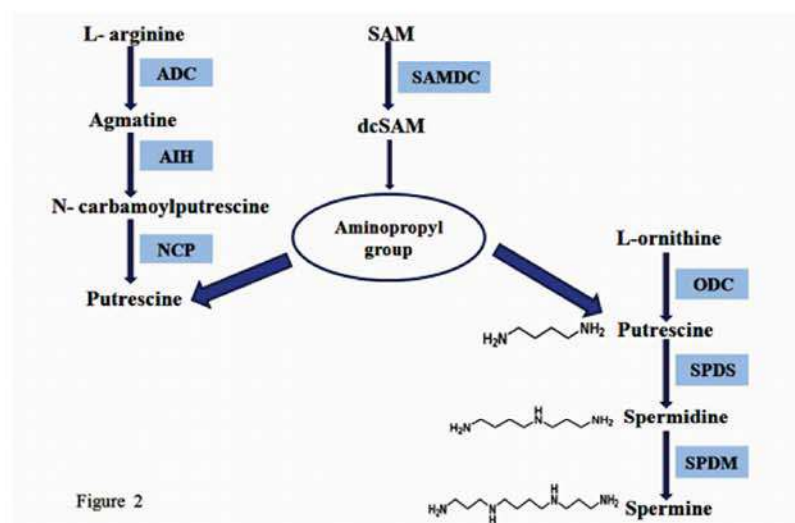


Figure 2

### Proline-polyamine combined pathway

From the two preceding paragraphs, it is evident that both proline and polyamine have a common precursor, orni-

thine. It is reported that the treatment of ornithine under saline conditions increased the amount of proline content in tobacco cells along with the level of the putrescine and antioxidant capacities of the cell. However, it did not show any effect on spermidine levels or protection of the cells against membrane lipid peroxidation<sup>38</sup>. In wheat leaves, it was seen that proline accumulation showed direct correlation with putrescine content but not with spermidine content<sup>39, 40</sup>. It was observed that if Put level was increased (either by pre-treatment or by increasing the ABA level) there was an increase in the activity of P5CS gene. In this context, it was also seen that ABA also plays some role here because only ABA pre-treatment increased the level of *oat* gene which synthesises the enzyme ornithine aminotransferase, which is required in the pathway of proline biosynthesis from ornithine. Increase in ABA hormone stimulated the activities of both *p5cs* gene and *oat* gene whereas increment in Put level increased only the *p5cs* gene activity.

Proline and polyamine have another common precursor, which is glutamate. Therefore, it can be postulated that a significant change in the polyamine pool can cause a shift between the synthesis pathways of proline and polyamines. Proline biosynthesis is also affected if more ornithine is diverted to PA biosynthesis. It was seen that P5CS transcript level was in a negative relation with SPM content but in a positive correlation with ABA levels and also with the gene expression levels of ADC, NCED and SAMDC. Whereas the OAT transcript level was in a positive relationship with ABA level but varied negatively with SPM content<sup>41</sup>.

### Approaches to develop transgenic salt-tolerant rice

Thus we see that same compounds can influence the biosynthesis of proline and polyamines and their effects regulate the accumulation of proline and polyamines within the cell. It can happen that overexpressing any such regulating compound in the cells of salt-stressed rice plants may increase the levels of both proline and polyamines and thus reduce the detrimental effects of salt stress more effectively. One such example exists where the *adc* gene from *Datura stramonium* was overexpressed in rice, and it conferred extra capability to withstand drought stress to the rice plants. Due to this overexpression, the putrescine level was high, and thus the higher polyamines like Spm and Spd were synthesised in higher amounts thus contributing to enhance stress response<sup>42</sup>. Previously we saw that increased Put level enhanced the activity of the gene *p5cs* which has a role in proline synthesis. Thus, it may be that with increased polyamine synthesis increased proline accumulation may also have played a role in increased stress response in the rice plants. Glutamine synthase (GS) an important enzyme in nitrogen metabolism plays a significant role in synthesising the amino acid glutamate. It has been found that rice plants overexpressing GS1 and GS2 gene have enhanced stress tolerance ability<sup>43</sup>. There are various instances stated in this article which indicate that GS plays a role in the accumulation of both proline and polyamines under stress condition in plants. Tobacco plants with downregulated *samdc* gene showed reduced vigour under salt stress when compared with wild type<sup>44</sup>.

### Need for candidate genes

Hence, it is evident that many of the enzymes like arginine decarboxylase (ADC), glutamate synthase (GS), S-adenosylmethionine decarboxylase (SAMDC) can regulate the synthesis of proline and polyamines. Therefore, we can say that the genes encoding these enzymes are the candidate genes in proline-polyamine combined pathway. However, further works are required to know precisely the effects of overexpressing or silencing the genes of the other enzymes in the pathways of proline and polyamine biosynthesis, and if any significant contribution is found, then those genes should be validated accurately.

### Looking for different allelic forms of the candidate genes

There are few reports of overexpressing some of the candidate genes in rice from other sources providing enhanced stress tolerance to rice plants<sup>42</sup>. An alternative approach, which is being implemented by many workers, is the identification of QTL/QTN related to complex traits in natural populations instead of bi-parental populations<sup>45</sup>.

Twenty SNPs associated with the Na<sup>+</sup>/K<sup>+</sup> ratio has been identified using 220 rice accessions. These SNPs contributed to about 5-18% of the phenotypic variance observed between the different rice varieties<sup>46</sup>. In another study, 22

candidate genes associated with different salinity tolerant phenotypes have been identified in Indian rice germplasm; additionally, their haplotypes have also been worked out<sup>47</sup>. Naveed et al.<sup>48</sup> identified various QTN and candidate genes related to salt tolerance at the germination and seedling stage in rice by screening 208 rice mini-core accessions from 25 countries. They used GWAS technique and found 6 QTN contributing salinity tolerances at the germination stage and 14 QTN contributing to salinity tolerance at seedling stage. Altogether they identified 22 candidate genes and their allelic forms in the landraces through bioinformatics and haplotype analyses<sup>48</sup>.

Singh et al.<sup>49</sup> found that the two salt stress-related genes, SOS1 and SKC1 showed the highest haplotype diversity among 69 diverse Indian rice landraces. In another work, 11 significant SNPs related to salinity stress among 392 rice accessions were identified, and the consequences of these SNPs were traced up to protein level. This work also identified 40 different allelic variants of 5 genes associated with salinity stress tolerance<sup>50</sup>.

Thus we see that rice landraces have natural allelic variants of many candidate genes conferring different responses to combat salinity stress. Expectedly, these landraces harbour different forms or alleles of those candidate genes regulating both proline and polyamines level under abiotic stress. In this context, salt stress-specific SNP (Single Nucleotide Polymorphism) markers can be used for large-scale genotyping of the hitherto unexplored landraces capable of growing in saline soils. In this way, a haplotype can also be prepared for the specific salt stress specific SNPs in different rice landraces. It will help to progress the MAB (Marker Assisted Breeding) programme for improved rice germplasm with enhanced salt tolerance.

## Conclusion

In this article, we reviewed that how proline and polyamines play the role to reduce the harmful effects of salt stress and also other abiotic stresses. Therefore, overexpressing such candidate genes which can enhance the levels of both proline and polyamine simultaneously may prove to reduce the ill-effects of salinity. Consequently, the salt-susceptible rice varieties will be able to withstand salt-stress more efficiently. However, it remains to be seen whether proline and polyamine act antagonistically to each other and also whether they individually cause any ill-effects on the rice plant. Since the interdependence of proline and polyamines in alleviating salt-stress has not been explored much, so information regarding their antagonistic effects (if any) could not be reviewed. The most important part is the identification of candidate genes of the proposed pathway. Also finding out allelic variants of the candidate gene is a daunting task as information about the landraces is limited. Thus, exploring a new path for developing salt-tolerant rice overexpressing the proline-polyamine biosynthetic genes may prove fruitful.

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