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Nature's marvel, Deep water rice

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ABSTRACT

Rice is the most important food crop of India. It is the staple food in most of the North Eastern parts as well as Southern part of India. Therefore, the people of North East India especially Assam is completely dependent on rice cultivation and its production. However, Assam is immensely affected by flood during the monsoon season. During that time of the year, most of the rice varieties are intolerant to water stress by submergence. Therefore, farmers are only able to cultivate deep water rice varieties during this season. Deep water rice shows an incredible tolerance to water-logged condition by escaping or managing to survive during submergence. Due to its fascinating water stress tolerance properties, it has been a major topic of concern. This review aims at encompassing the major studies done to investigate the factors responsible for making the plant tolerant to submergence and to evaluate its phytochemicals and nutritional values. Studying deeper into the plant can help in its biofortification as well as improve its yield through different breeding programs using enhanced varieties and thereby develop its marketing status.

Key words: Anthocyanin, Antioxidant, Biofortification, Deep water rice, QTL (Quantitative Trait Loci), Water stress tolerance

INTRODUCTION

Rice (*Oryza sativa*) is a semi-aquatic crop plant that is adapted to a broad range of aquatic conditions, as well as aerobic soil of uplands to anaerobic or water logged fields in lowlands of flood prone areas (Miro and Ismail, 2013). It is one of the most significant food crop upon which more than a third of the world's population is dependent on. India is considered as the world's second largest producer of rice after China (Mohanty *et al.* 2013). Rice is the most important food crop which is consumed by half of the world's population everyday as a primary food source which provides 20% of the world's dietary energy supply whereas wheat and maize contributes 19% and 5% respectively (Bin Rahman and Zhang, 2023). Rice production in India has surged from 54 million tons in late 1960s to more than 178 million tons in 2020 (Modi *et al.* 2022). Assam is one of the major rice producing states of India. Due to adaptations to the various agro-climatic conditions in different regions of the state, a significant diversity in the rice varieties is seen from hill slope rice cultivars to deep water rice cultivars. Although Assam is bestowed with varieties of rice strains, however it suffers from heavy rainfall from the month of April to September. During this time of the year, most of the regions get submerged due to flood and cultivation of the rice varieties is not feasible. Deep water rice (DWR) varieties serve as a blessing to the local communities (Rohilla *et al.* 2023). Cultivating DWR is the only means of subsistence for most of the people during this time. Therefore, deep water rice has great agronomic importance in many densely populated areas where no other crop can be grown during heavy rainfall of monsoon. Deep water rice cultivation is practiced mainly in river basins of the Ganga and Brahmaputra of India as well as in Bangladesh, Burma, Vietnam, Cambodia and Thailand (Cattling, 1993). In Asia, it is grown from latitude 27º N in Assam and Uttar Pradesh of India, to latitude 3º S in Indonesia (Puckridge *et al.* 2001). In Assam, DWR is commonly known as Bao dhan (Rohilla *et al.* 2023). Around 100,000 ha of land area is under deep water rice cultivation. This area is covered by Upper Assam districts such as Dhemaji, Lakhimpur, Sibsagar and Majuli whereas some districts belonging to Middle and Lower Assam such as Kamrup, Nalbari, Barpeta, Goalpara and Morigaon. In case of Dhemaji, around 25,000 farm families belonging to 25 Panchayats rely on deep water rice cultivation (Rohilla *et al*. 2023). Even though not properly

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listed, it is estimated that there are more than 70 landraces of Bao dhan in Assam (Loying *et al.* 2010). Apart from Assam, deep water rice is also cultivated in Bihar, Uttar Pradesh, West Bengal and Orissa (Rohilla *et al*. 2023).

DWR varieties are basically grown in lowland and flood-prone conditions. This is possible due to the brilliant adaptation of DWR to water-logged conditions. Due to its interesting nature and captivating adaptation to water logged conditions, it has been studied worldwide and many researchers have attempted to identify the factors responsible for making it flood resistant. This review focuses on the various directions in which different studies such as physiological, molecular, as well phytochemical investigations that were carried out on deep water rice in different parts of the world to study the flood tolerant properties as well as nutritional values. Researchers have carried out studies on the various techniques adopted by deep water rice varieties to sustain during water-logged condition, they found that certain QTLs (Quantitative Trait Loci) are responsible for internode elongation that gives the DWR varieties the ability to escape submergence and raise the green foliage part above the water level and perform photosynthesis in order to provide nutrition to the whole plant and keep the plant alive during flood seasons. It was also reported that ethylene is also responsible for the elongation of the internodes through gibberellin (Fukao and Bailey-Serres, 2008; Minami *et al.* 2018; Sun *et al.* 2022). Moreover, it was successfully established that introduction of QTLs responsible for flood tolerance found in deep water rice varieties into non-deep water rice can transform them into tolerant ones and can sustain submergence as well.

Apart from its deep water stress tolerant ability, DWR varieties are bestowed with many nutritional values. Some DWR varieties of Assam such as Jul Bao and Negeri Bao was reported to contain comparatively higher amount of protein than other rice varieties (Rohilla *et al.* 2023). They are also rich in iron, zinc, vitamins as well as minerals. Deep water rice with red kernels has significant antioxidant properties due to presence of high amount of anthocyanin, the pigment which is responsible for the dark reddish pigmentation of the kernels. Deep water rice has been a subject of importance due to its amazing submergence tolerance capacity as well its other qualities. Therefore, various studies have been carried out to know more about the plant and what it has to offer for human welfare. The major focus of most of the researches on rice carried by several rice research institutes and universities is to improve the yield and meet the requirements of the growing population (Bin Rahman and Zhang, 2022). This review aims in reflecting some of the major studies done on DWR.

MORPHOLOGICAL AND ANATOMICAL STUDIES

Elongated internodes

Deep water rice is known for long internodes which help the plant to escape the submergence during flood conditions. The deep water rice varieties can overcome water-logged conditions during two types of flood *i.e.,* flash flood and deep water flood by adopting two different mechanisms. During flash flooding, some DWR varieties undergo quiescent stage during which the plants are able to sustain complete submergence and stops growth during the submerged condition for few weeks until the water subsides. After the water level drops, the plant starts to grow again and will start elongating. During this stage of growth, the plants will stop the metabolic processes in order to save energy. This strategy is termed as the “quiescent strategy”. During deep water flooding conditions which lasts for months where the water level ranges from 50cm to about 4m, some deep water varieties will escape submergence by promoting rapid internode elongation which will avoid the foliage from getting submerged. In this way, the parts above the water level will be able to perform photosynthesis as well as respiration and will keep the entire plant alive. This strategy is termed as the “escape strategy”. It was seen that despite the two contrasting behavior, the chief mechanism is controlled by one type of transcription factor involved in the biosynthesis of ethylene and gibberellic acid (Nagai *et al.* 2010).

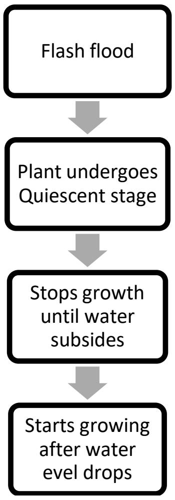

Figure 1(a). The Quiescent strategy

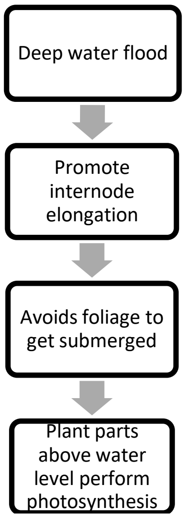

Figure 1(b). The Escape strategy

Adventitious roots

Deep water rice shows emergence of adventitious roots in submerged condition. Presence of these secondary roots, adventitious roots from the nodes can take over and support the primary root system that is susceptible to become dysfunctional due to limited oxygen supply in submerged condition (Steffens *et al*. 2006) This emergence of adventitious roots is mainly regulated by ethylene that gets accumulated in submerged plant parts. Gibberellic acid also plays role in a synergistic way but is ineffective alone. Whereas another phytohormone, abscisic acid functions as a competitive inhibitor of gibberellic acid as well as an inhibitor of ethylene signaling pathway (Steffens *et al.* 2006).

Panicle

Studies on the phenotypic diversity in panicle and nutritional value of ten commonly cultivated indigenous deep water rice varieties of Dhemaji district revealed Dal bao to be most nutritious with superior panicle traits (Dutta and Borua, 2011). The ten varieties taken were Panikakuwa, Kakuwa, Mia bao, Dal bao, Maguri bao, Negheri bao, Panidhan, Bhu bao, Amana and Happy bao.

Aerenchyma

Deep water rice along with other submergence tolerant rice varieties, the number of roots per stem is comparatively higher than other varieties. The roots of deep water rice and other wetland species has aerenchymatous tissues, whose amount increases upon exposure to water-logged conditions. Ethylene serves as an important signal that triggers the lysigenous aerenchyma formation in rice plants during soil flooded condition (Fukao and Bailey-Serres, 2008; Sauter, 2013). The presence of aerenchyma provides a low resistant internal pathway for movement of oxygen within the roots (Colmer, 2002).

PHYSIOLOGICAL STUDIES

Role of ethylene and gibberellin

A correlation was seen among the three phytohormones ethylene, gibberellic acid (GA), and abscisic acid (ABA) and their role in internode elongation of deepwater rice. At the time of submerged condition, the reduced diffusion of ethylene leads to its accumulation, which then stimulates the ethylene signaling cascade, leading to the depletion of the concentration of ABA and the increase in content of GA (Fukao and Bailey-Serres, 2008). During submergence, deep water rice is capable to overcome submergence by adopting certain responses by which they are able to project out their photosynthetic parts above water level into the air. This escape strategy is adopted as a result of high production and accumulation of ethylene (Fukao and Bailey-Serres, 2008). Thus, in submerged condition, accumulation of ethylene triggers several acclimation responses including shoot elongation, adventitious root formation, aerenchyma formation, carbohydrate metabolism as well as ethanolic fermentation (Fukao and Bailey-Serres, 2008).

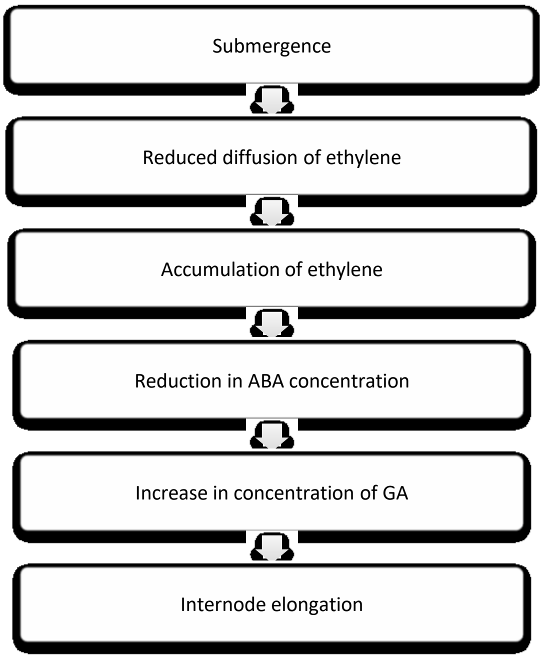

Figure 2.: The cascade of events triggered by submergence in DWR

Jasmonic acid

Jasmonic acid metabolism also appears to be involved in the submergence-mediated internode elongation response of DWR varieties (Minami *et al.* 2018). It was found that ABA is a negative regulator of internode elongation during submerged conditions (Hattori *et al.* 2009). Different morphological as well as physiological characters were studied in deep water varieties like Amona, Kakua, Rangoli, Panikakua, Maguri bao, Deori bao, Moimonsingia, Dal bao, Duatkalam, and PJNB-96-10 of Majuli. Out of these varieties, Maguri bao was identified to have better and more desirable physiological traits and can be included in deep water rice breeding programs (Baruah *et al.* 2006).

MOLECULAR STUDIES

QTL Analysis

During 1980s, rice scientists failed to successfully combine submergence tolerant and high grain yield through conventional breeding. However, in the1990s, rice scientists found that, in certain rice varieties, submergence tolerance is controlled by a single major quantitative trait locus (QTL), which was termed *SUB1*. In early 2002, through marker-assisted backcrossing (MAB), the *SUB1* QTL was successfully introgressed, that is, a hybridization technique in which one gene is transferred from one plant to another through breeding, not through genetic modification, into a popular Indian rice variety, called Swarna. Swarna was selected because it is one of the most popular rice varieties in the region and, as a consequence, farmers might be more inclined to adopt Swarna-Sub1 (Yamano *et al.* 2013). Although deep water as well as non-deep water rice varieties both have the same machinery for internode elongation, however, non-deep water rice varieties cannot activate needed responses to sustain flood while deep water rice varieties easily can because of the major QTLs they harbor to grow even in submerged conditions (Hattori *et al.* 2007), also after genetic analysis it was revealed that these major QTLs are located on the long arm of chromosome no. 12 of deep water rice cultivars (*Oryza sativa*) (Hattori *et al.* 2007).

Two deep water varieties were used one from Bangladesh, C9285 and one wild variety from India, W0120 which have deep water characteristic along with them, one non-deepwater rice cultivar, Taichung 65 (T65) was used as a control and as a crossing material. Then NIL, harboring the traits, were produced by crossing C9285 and T65. The F1(T65/C9285) were backcrossed four times with T65 using marker assisted selection, likewise three NILs were developed. Three parameters were selected for evaluating internode elongation *viz.* total internode elongation length (TIL), number of elongated internode (NEI) and lowest elongated internode (LEI). The progenies of the NILs expressed the presence of the traits supporting internode elongation under deep water condition. Also the progenies of the NILs were used to map the QTLs and the most effective QTL was located. The comparison of the locus of the most effective QTL was carried out between the two cultivar, C9285 and W0120. After the comparative study on the location of the most effective QTL, it was found to be located on the common locus in chromosome 12 in both the cultivars and that in water-logged condition, this QTL is most crucial for internode elongation (Hattori *et al*. 2008). Haplotype network analysis of 18 eQTLs identified as to be related to internode elongation has been investigated in 92 deep water landraces, one IRRI cultivar and one African landrace, the analysis categorized all the genotypes into different haplotypes based on SNPs related to internode elongation specific eQTLs.

Expansins

Expansins are proteins that mediate long-term extension of isolated cell walls. Alpha expansins show less activity in grass cell walls compared to their activity in dicots and monocots such as maize and rice contain more numbers of putative beta-expansin genes than does, for example, Arabidopsis (Cho and Cosgrove, 2000). They are grouped into two related families, the alpha and beta-expansin (Lee and Kende, 2001). Beta-expansin genes were located and found to be responsible for rapid internode elongation of deep water rice varieties and that the expression of the expansin genes were induced by gibberellin and wounds (Lee and Kende, 2001).

SNORKEL genes

The effect of ethylene in stem elongation is highly significant. During deep water conditions, ethylene gets accumulated in the plants and this rise in ethylene concentration induces the expression of two genes SNORKEL1 and SNORKEL2. The products of this two genes regulated deep water response and as a result internode elongation takes place via gibberellin which helps the plants to escape submergence. These two genes were found to be absent in non-deep water rice variety (T65, a non-deep water rice variety taken in the experiment) (Hattori *et al.* 2009).

Figure 2: Effect of SNORKEL genes in deep water condition

PHYTOCHEMICAL STUDIES

Anthocyanin

Bao dhan or deep water rice is a complete organic crop having high nutritional values compared to other rice varieties (Rohilla *et al.* 2019). Anthocyanin is a polyphenolic pigment found in flowers, fruits, vegetables and leaves. Deep water rice kernels are purplish due to the anthocyanin which has antioxidant, anti-inflammatory, anti-cancer as well as anti-microbial properties in vitro. The research was conducted on five landraces *viz.* Dal, Surabhi, Kahi Jool, Kekua and Buruli of deep water rice grown in flood affected areas of Assam and another variety Chakhao was collected from Manipur. Out of these, Surabhi was the darkest red amongst the 6 varieties (Kalita and Handique, 2021). In brown-red rice of Thailand, the total polyphenol content (TPC) was recorded to be found in the range of 1.018-0.069mg GAE g^{-1}. Earlier studies proposed that pigmented rice has more TCP as compared to non-pigmented rice (Chen *et al*. 2012). The importance of red kernelled deep water rice came into light when it was found to be a nutritionally rich rice variety with antioxidant property due to high amount of anthocyanin (Loying *et al.* 2010). Since anthocyanin is found to be a very efficient antioxidant, therefore the red rice having high amount of anthocyanin is nutraceutically rich as well (Kalita and Handique, 2021).

Starch

Rice is the chief dietary energy for many countries specially India. Rice provides a good source of dietary energy mostly because of starch which makes up 90% of milled rice on a dry weight basis. Starch is the major component of rice and accounts for more than 80% of the total constituents. It is also an important determining factor of grain quality particularly cooking as well as eating features of the grains. (Patindol *et al.* 2015). The two main components of starch are amylose and amylopectin. The content of these two components determines whether the rice after cooking becomes sticky and gelatinous or non-sticky. Sticky rice contains more amylopectin and very negligible amount of amylose where non-sticky rice will have more amylose than amylopectin and the grains remain separate after cooking. A comparative field study between deep water tolerant and intolerant varieties specified that seedlings belonging to tolerant species contained 20-30% more starch as compared to the intolerant varieties. These reserved starch were consumed rapidly amidst submergence which were used up for ATP production during germination, due to which they display rapid growth in order to keep up with the water level and escape submergence (Sauter, 2000).

Protein

Seed protein profiling is used to detect the variations in different deep water landraces of Assam (Loying *et al.* 2010). Among ten indigenous DWR landraces *viz.* Negheri, Jool, Bam-Kokua, Kolioi, Ranga, Gotha, Do-kokua, Adolia, Bawla and Holodhar which were collected from flood affected areas of Bokakhat and Dergaon sub-divisions of Upper Assam were taken among which Ranga, Holodhar and Adolia had white kernel and while the rest seven had red kernel and Negheri being the darkest among them. After conducting crude protein content analysis on these varieties, it was found that Do-kokuwa had the highest protein content. Seed protein profiling revealed that Bawla is quite different from the rest landraces. It further showed that Adolia and Holodhar are identical at molecular level. Similarly, Gotha and Kolioi have no significant difference at molecular level (Loying *et al.* 2010). From nutritional view point, the most promising aspect of deep water rice is its high protein content. In general protein content of rice vary from 6% to 14% with an overall mean of 10.5% (Baruah *et al.* 2006) working with ten indigenous land races of deep water rice of Assam found crude protein in the range of 8.03 to 13.20% with a mean of 11.78%.

Iron

Generally grains of deep water rice landraces, Basmati genotypes are rich in iron content (Ravindra, 2013). A study conducted in Majuli, Assam on ten deep water rice varieties, *viz.,* Amona, Maguri, Rangoli, Kakua, Panikakua, Deoribao, Moimonsingia, Duatkalam, Dalbao and PJNB-96-10 revealed that Duatkalam has the highest iron content.

ANAEROBIC GERMINATION

Floods causing submergence may result into hypoxic or anoxic a condition which cuts down aerobic respiration in germinating seeds and leads to anaerobic respiration that result into low ATP production. During such condition, the capacity to germinate and initiate the growth of the seedling is of preferable quality. Such a property to germinate not only in submerged condition but in anoxic condition is uncommon in cereals (Magneschi and Perata, 2009). With the combination of GWAS (Genome-Wide Association Study) and transcriptomics, 77 potential gene candidates were identified related to anaerobic germination tolerance which was studied in a geographically diversified rice panel of accessions 241 (Thapa *et al*. 2022). A QTL study was conducted to understand the genetic basis of tolerance of anaerobic conditions during germination using a population which was derived from a cross between IR42, a susceptible variety to anaerobic condition, and Ma-Zhan Red, a tolerant landrace of China. The dry seeds were sown under 10cm of water and then phenotypic data were collected based on the survival rates of the seedling at 21 days after sowing. QTL analysis of the mapping population identified six major QTLs that were located on chromosomes 2, 5, 6, and 7 (Septiningsih *et al.* 2013). During water logged conditions, several problems develop within the root zone especially during germination. Low oxygen supply causes reduction in root development that makes the developing seedling unable to take up adequate water and nutrients. In addition to that during such conditions, several phytotoxic compounds start to accumulate to toxic levels such as reduced iron, (Fe^{+2}), manganese (Mn^{+2}), hydrogen sulfide (H_2S), and intermediates of anaerobic carbon metabolism, like organic acids. However, deep water rice varieties are seen to be able to germinate during such condition by coleoptile elongation during germination that helps the seedling to grow and raise the shoot above anaerobic condition into an aerobic surrounding (Miro and Ismail, 2013) and the plant is able to emerge out of the water level and continue to grow.

PLANTING VARIABLES

Planting variables also play a crucial role in productivity of the crop. In an experiment, five deep-water rice cultivars Bhagirathi, Sabita, CN-1231-11-7, Jaya cross and CN-1039-9 were studied for varietal performance in Kharif season in West Bengal and four planting variables were taken – S1 (20cm × 15cm spacing with 5 seedlings), S2 (20cm × 15cm spacing with 3 seedlings), S3 (30cm × 20cm spacing with 5 seedlings), S4 (30cm × 20cm spacing with 3 seedlings). The aim was to find out the best planting variable and to identify the best performance among these five deep water rice varieties. After the completion of the experiment, CN-1039-9 variety among the five DWRs showed the best performance whereas, planting variable S1 (20 cm × 15 cm spacing with 5 seedlings per hill) recorded better growth features whereas planting variable S3 (30 cm × 20 cm spacing 5 seedlings per hill) documented better yield in semi-deep water situation in New Alluvial Zone of West Bengal (Tarafder *et al.* 2022).

Being bestowed with striking nutritional value along with its organic nature, deep water rice or Bao dhan seemingly has caught more attention in the global market. For instance, the United States based company, Lotus food Inc. has reported to have imported 70q of deep water rice from Dhemaji district of Assam via its partner company, Nature Biofoods, India Ltd, Haryana. The local demand of Bao dhan in Assam has increased from 3000q in 2012-13 to 12,000q recently (Rohilla *et al.* 2019). Nevertheless, a smooth and structured supply chain as well as collaboration between farmers and more entrepreneurs is needed for a successful commercial set out including both import and export of deep water rice to different parts of India and abroad. This will benefit the farmers greatly as they will gain high returns as the market for DWR is progressively rising (Rohilla *et al.* 2019).

CONCLUSION

Rice being a staple crop in many countries, biofortification of rice is a growing topic in the field of research and food technology to improve rice varieties and combat hunger. Deep water rice is a boon to rice cultivating areas which are affected by flood. This rice variety has adapted strategies to escape or survive deep water logged conditions. The morphological and anatomical characteristics such as presence of adventitious roots arising from upper nodes, elongated internodes, aerenchyma in roots has helped the plant to cope with submerged conditions. Major QTLs responsible for internode elongation were located in chromosome 12.A very significant relationship is seen between the phytohormone ethylene and genes such as expansin genes, SNORKEL genes *viz.* SNORKEL 1 and SNORKEL 2, whose interaction contributes in the elongation of internodes and helps the plants to extend their foliage above the water level and perform photosynthesis in order to keep the plant alive. Concentration of ethylene is seen to have a very important role in surviving flooded situations and the growth of internodes is induced by the interaction between phytohormones *viz.* ethylene, gibberellins and ABA. DWR can also germinate in anaerobic condition and the major QTLs responsible for this quality has been located successfully. Apart from its submergence tolerance capacity, deep water rice varieties are found to be endowed with multitude of nutritional values including protein content, iron as well as high content of anthocyanin with antioxidant properties. Several researches and breeding experiments have been undertaken to know more about this crop, to detect and quantify the presence of major nutrients and to compare the results with other non-deep water varieties of rice. DWR although being known for its tolerance to flood and high nutritional values, it is not a high yielding variety. However, there is still a need to do a thorough characterization of more varieties so that they can be incorporated in different breeding programmes for their biofortification and enhance their yield and marketing status.

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