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#### Chapter

### Present Status and Future Prospects of Drought Tolerance in Rice

Veerendra Jaldhani, Ponnuvel Senguttuvel, Bathula Srikanth, Puskur Raghuveer Rao, Desiraju Subrahmanyam and Durbha Sanjeeva Rao

#### **Abstract**

Rice is an important staple food crop across the world. It is mainly cultivated under irrigated lowland and also rain-fed upland conditions where drought stress is often noticed. Global climate change predicts an intensification of drought stress in future due to uneven rainfall which was witnessed for the last few years. Confronting drought stress can deliver fruitful crop returns in rice and scope for research extents. Drought stress affects the overall plant growth and yield. A prominent improvement has been made during last two decades in our understanding of the mechanisms involved in adaptation and tolerance to drought stress in rice. In order to achieve the marked crop returns from rainfed areas, there is a requisite of drought tolerant rice varieties, and genetic improvement for drought tolerance should be a prime area of concern in the future. A huge rice germplasm is available and good number of the germplasm possess drought tolerance and these genomic regions have been exploited in developing some drought tolerant rice varieties. The application of available genotyping methodologies, the identification of traits of interest, and key genetic regions associated with the drought tolerance have opened new prospects to successfully develop new drought tolerant varieties. This chapter deals with the importance of drought tolerance in rice crop followed by the evolution of molecular markers and breeding techniques in identifying drought tolerant QTL's/ genes and their utilization in the improvement of drought tolerant rice varieties.

Keywords: Rice, Drought tolerance, Markers, QTLs, Varieties

#### 1. Introduction

1

Rice is a staple cereal consumed by more than half of the world's population. It is cultivated in wide agro-ecological conditions including rain-fed conditions where drought stress is often evident due to erratic rainfall. Rice crop consumes around 3000 L water to produce 1 Kg rice. Drought stress is one of the major factors that leads to decreased rice production [1] and it can expand to above 50% of the global arable land by 2050 [2] due to loss of ground water, global climate change leading to decreased water sheds. Drought stress was noted in approximately 42 Mha of rice-producing area [3]. It is of two types, terminal and intermittent [4].

Drought stress is usually a dry condition where the water availability is less than a threshold level which causes damage to plants [5].

Lack of water for a long time leading to the death of plants is terminal drought while lack of water for a short time that leads to improper growth is intermittent drought [6].

Drought stress tolerance varies among the plant species and is defined as the ability of a plant to grow, develop and produce significant yield as well as economic benefit [7]. It is also defined as the ability of plant to survive at minimum water level in the fresh tissue (23%) [6].

Drought stress leads to morphological, physiological and biochemical changes in plants which ultimately lead to decreased yields. In response, plants synthesize reactive oxygen species (ROS), proteins and osmolytes to maintain turgor pressure. This osmotic adaptation provides dehydration tolerance to tissues [8–10]. But, this did not show yield benefit in rice [11].

The first landmark achievement in drought rice tolerance study was the identification of a small region on chromosome 8 homologous to chromosome 7 of wheat [12]. A marker based selection was proposed and this region was worked out to identify QTL's for vegetative leaf rolling and root traits- thickness and root to shoot ratio [13].

Historically, upland-adapted germplasm was of Japonica type while lowland-adapted germplasm was of Indica type [14]. In general, Japonica genotypes were dehydration avoidant while Indica types were dehydration tolerant. Geographically separated evolution and sterility problems have limited hybridization between the two types [15]. If breeding for both osmotic adjustment and rooting capacity is considered desirable, then the linkage between high osmotic adjustment and poor root traits needs to be broken.

RG1 QTL was identified while working with 52 recombinant inbred (RI) lines (F 7), a randomly sampled subset of a population originally developed to study the genetics of resistance to rice blast (*Pyricularia oryzae*) [16].

#### 2. First generation markers

#### 2.1 Restriction fragment length polymorphism (RFLP)

Rice RFLP maps developed at Cornell University [17, 18] and Japan [19, 20] is the basis of gene mapping research. In the 1988 wet season at IRRI, Co39 (maternal), a lowland, Indica cultivar developed in India, and Moroberekan, a traditional upland, Japonica cultivar originally developed in Guinea. Moroberekan is considered to be resistant to drought while Co39 is drought susceptible. About 50  $F_1$  seeds were obtained from the cross and only 15  $F_1$  seeds were randomly chosen and grown in a greenhouse to obtain an  $F_2$  population. About 300  $F_2$  seeds were randomly selected and planted in the Rapid Generation Advance (RGA) [21] greenhouse from  $F_2$  to  $F_6$  using single seed descent (SSD). All panicles were bagged at each generation and  $F_7$  seeds were used for genotype analysis [16].

DNA was extracted from the leaves of the two parents and digested with restriction enzymes DraI, EcoRI, EcoRV, HindIII and Scal. The digested DNAs were electrophoresed on 0.9% agarose gels and transferred to Hybond N<sup>+</sup> membranes (Amersham Corp., Chicago) according to the manufacturer's instructions. 280 DNA clones distributed throughout the 12 chromosomes of rice (184 rice genomic clones, coded RG; 62 rice cDNAs, coded Rz; and 28 oat cDNAs, coded CDO) were linearized and labeled with <sup>32</sup>PdCTP by the random hexamer method [22]. Hybridized filters were washed once in 1.5 X SSPE and once in 0.5 X SSPE at 65° for

15–20 min. Filters were exposed to X-ray film  $-80^{\circ}$  at with one intensifying screen for 1–4 days. 127 informative probes were used for segregation analysis of the RI lines using the procedures outlined above. Mapmaker [23] and Map Manager [24] were used to establish the RFLP map.

Since the *indica* × *japonica* crosses suffer from sterility, distorted segregation, etc., [16], breeders chose to work on the accessions of *indica or japonica* sub-species, RFLP was considered laborious over RAPD which gained popularity among researchers [25].

#### 2.2 Random amplified polymorphic DNA (RAPD)

Prior to the availability of complete rice genome sequence, RAPD markers were useful in developing drought tolerant varieties. It is the simplest, cheaper, sensitive and useful technique for the genotype identification, population and pedigree analysis, phylogenetic studies, genetic mapping [26] and analysis of genetic fidelity of commercially micropropagated plants [27]. It is simple since it requires less DNA and simultaneously doesn't require southern blotting and radioactive labeling [28]. Considering the usage of RAPD in wheat, maize, pea-nut, broccoli and cauliflower, RAPD was applied in rice to detect diversity in the low land and upland rice varieties with an aim to identify drought-resistant loci [29] as given below.

Thirteen rice cultivars were grown in a growth chamber at  $28^{\circ}$ -day temperature,  $25^{\circ}$ -night temperature and with an irradiance of  $800 \, \mu moles \, m^{-2} \, s^{-1}$  for  $12 \, h$  a day. At six leaf stage, leaves were collected and stored in liquid nitrogen and genomic DNA was extracted and purified [22]. Forty-two GC-rich 10 bp random primers were used as RAPD markers. DNA amplification was done in a PCR (Perkin Elmer Cetus) programmed for 45 cycles of 1min at 94°C,1 min at 37°C and 2 min at 72°C. The reaction conditions include 25  $\mu$ l total reaction volume having 10 mM Tris-HC1 (pH 8.3), 50 mM KC1, 2mMMgC1<sub>2</sub>, 50 mM each of dNTP's, 10 ng of a single random primer, 25 ng of genomic DNA and 2 units of Ampli Taq DNA polymerase (Perkin Elmer Cetus) and 50 ml of sterilized mineral oil.

At the end of amplification, 10 µl of each amplification mixture was loaded in either 1.4% agarose (0.5 to 4 kb) or 5% polyacrylamide (<500 bp) gels for electrophoresis in 1 x TBE (89 mM Tris, 89 mM boric acid and 2 mM EDTA). Gels were stained with ethidium bromide and photographed under UV light. Pair-wise comparisons of genotypes, based on the presence (score 1) or absence (score 2) of each marker similarity coefficients were calculated which were used to construct UPGMA tree. One-to-twelve DNA amplicons were observed from each genomic DNA sample. A total of 260 DNA fragments were amplified and 208 (80%) of these showed polymorphisms. Upland cultivars and lowland cultivars were classified into two main clusters-*japonica* and *indica* with seven (Azucena, Rikuto Norin 21, Moroberekan, IAC25, IRAT13, OS4, and 63–83) six (BPI-76 NS, IR20, IR36, CO39, MGL-2 and Salumpikit) cultivars respectively. Later, they screened 2074 rice varieties for drought tolerance where upland varieties were identified with higher score for drought tolerance and were recommended for donors for breeding drought tolerant varieties as well as for developing molecular markers.

However, like RFLP, RAPD is also disadvantageous due to low polymorphism among *japonica* rice, need for hundreds of markers to locate markers in the QTL and absence of RAPD markers for some regions of the chromosomes [30].

#### 2.3 AFLP (amplified fragment length polymorphism)

Restriction enzymes were reported from bacteria. This enzyme identifies foreign DNA in bacterial cells based on the target site and generally, cuts the DNA within

this site if the site is un-methylated. Eventually, the broken DNA cannot show its effect on the host bacterial cell and hence, the natural function of restriction enzymes is to restrict the growth of foreign DNA. Restriction enzymes differ in the length of their target sites (4–8). It is universally accepted that the probability of finding the smaller length of sequence is manifold higher than longer sequences i.e., in other words if the length of the target site is less, then, it can be repeated more frequently in the DNA. In the above example, a 6-base target is rarer to find than the 4-base target site. Adapter /adaptor/linker is a short, chemically synthesized (known sequence), single-stranded or double-stranded oligonucleotide that can be ligated to the ends of other DNA/RNA molecules to convert them to sticky ends of desired sequence.

DNA was isolated from 80 plants of F<sub>2</sub> population of the cross 'Labelle' × 'Black Gora' [30] and AFLP was conducted [31]. AFLP involves cutting of genomic DNA with two restriction enzymes that need different lengths of target sites, a 6-base (or "rare") cutter (EcoRI) and a 4-base (or "frequent") cutter (MseI), ligating adapters to the fragment ends, amplifying MseI-EcoRI fragments with primers that match the adapter and contain additional selective nucleotides at the 3' end and separating the fragments on denaturing polyacrylamide gels. EcoRI and MseI adapters, T4 DNA ligase, DTT and water were added to the restriction reaction-mixture which was incubated for 3 h at 37°C. Then, preamplification of DNA was done with primers which were labeled subsequently with spermidine. The amplicons were separated on 4.5% denaturing (urea) polyacrylamide gels for 90 to 120 min at 120 W. The gels were dried and exposed to X-ray film for 4–7 days.

Bands showing clear polymorphism were scored as present ("1") or absent ("0"). Genetic similarity was computed as the number of common bands divided by the total number of bands of both accessions, and genetic distance was computed as 1 minus this value [32]. These distances were used to construct cluster diagrams by UPGMA method (SAS, PROC CLUSTER) [33]. AFLP is cheaper than RAPD. AFLP is useful to screen small number of samples with large number of markers.

#### 3. Second generation markers

#### 3.1 Inter simple sequence repeats (ISSR)

ISSR markers are well distributed in the eukaryotic genome [34] more feasible and reproducible than RAPD [35] highly polymorphic, less expensive and independent of sequence information [36]. Polymorphism of 73.02% with RAPD markers and 90.91% with ISSR markers was observed between six rice lines [37]. Seventeen ISSR primers- (8 based on (AG)<sub>8</sub>, 8 on (GA)<sub>8</sub>, and 1 on (GATA)<sub>4</sub> were used to screen 12 cultivars as presented below [38].

Genomic DNA was isolated from freshly harvested young leaves of each cultivar by Mini prep method. ISSR-PCR was conducted and the amplified products were resolved in 1.8% agarose gel in the presence of ethidium bromide and were documented under ultraviolet light. Polymorphism information content (PIC) was calculated based on the presence (score 1) or absence (score 0) of band. Similarity scores were calculated and un-weighted pair-group method with arithmetic average (UPGMA) dendrogram was generated sub-program of NTSYS-PC software version 2.0at 1000 bootstrap. The three drought tolerant varieties formed one sub-cluster by (GA)8YG primer.

#### 3.2 Simple sequence repeat (SSR) and SNPs (third generation markers)

Rice crop was recognized with more than 20,000 SSR markers and over one million SNPs and Indels, which include both functional and non-functional markers [39–41]. This has opened up huge opportunities for the use of molecular markers in diversity analysis, mapping genes/QTLs for various agronomic traits under drought, and their use in marker-assisted breeding (MAB) and also in positional cloning of QTLs to identify candidate genes for complex traits [42].

The accessibility of complete rice genomic sequence information, rice linkage maps, and molecular marker technology has made it possible to dissect complex traits into individual quantitative trait loci (QTLs) [43-46]. Linkage mapping, association mapping, nested association mapping, marker-aided recurrent selection (MARS), and genome-wide selection (GWS) are different approaches currently followed for the mapping and introgression of QTLs for drought tolerance. There are several sources of genomic variation such as QTL main effects, QTL × QTL interactions, and QTL × environment interactions. A thorough understanding of this variation is very important before embarking on marker-assisted selection (MAS) of drought QTLs. Several major-effect QTLs for grain yield under drought have been identified and are being used in marker-assisted breeding/ pyramiding. Courtois et al. [47] identified the meta-QTLs for root traits under drought stress. Meta-analysis of 53 grain yield associated QTLs identified from 15 previous studies bring about in 14 meta-QTLs. In general, rice varieties with deep and high-volume root system exhibits better adaptability under drought conditions [48]. Among the root traits, root length, volume, thickness and root growth angle (RGA) are playing key role in mitigating drought tolerance [49]. In turn, RGA determines the root depth. The deeper and profuse root architecture support plants to extract water from deeper soil layers. The three major QTLs governing root growth angle (RGA) in rice were reported by Uga et al. [49–51]. Among them, DEEPER ROOTING 1 (DRO1) is the significant one and this QTL has been fine mapped and the underlying gene, an early auxin responsive factor, has been cloned using IR64, a shallow rooted variety and Kinandang Patong (KP), a deeply rooted variety. The variation in the DRO1 gene in the major Indian rice genotypes used in drought tolerant breeding plans and their association with RGA is reported [52]. So far, 675 root QTLs and more than 85 genes related to 29 different root parameters have been reported in rice [47]; https://snp-seek.irri.org/). The introduction of deep rooting traits in high yielding varieties is a resourceful way of enlightening drought tolerance in rice.

#### 4. Tissue culture

Polyethylene glycol (PEG) can decrease moisture in tissues [53] by osmosis and eventually reduce callus growth. Manually dehusked brown rice of four rice varieties- Pusa Basmati 1, Taraori Basmati, Pant Sugandh Dhan 17 and Narendra 359were washed with detergent (teepol), washed with sterile distilled water, surface sterilized by 70% ethanol followed by 1% sodium hypochlorite and 0.1% mercuric chloride and were inoculated on to MS medium [54] having 30% sucrose and 8% agar. One month old calli were transferred onto the MS medium having a series of concentrations (0 (control), 10, 20, 30, 40, 50, 60 and 70 g/L) of PEG. After 30-day incubation, healthy calli at 70 g/L PEG were identified as drought tolerant and were subjected to shoot and root inductions separately followed by the development of plant lets. Loss of moisture content was least in Narendra 359 (2.99%) and

highest in Pusa Basmati 1 (20.64%) and it indicates the drought response variation among the rice genotypes. Proline content in calli of all varieties increased with the increase in PEG concentration [55].

#### 5. Gene expression

Transcriptomic analysis identified stress responsive genes like transcription factors, genes encoding for osmolyte production, reactive oxygen species (ROS) scavenging and other metabolic pathways etc. which help in developing drought tolerant varieties [56, 57]. They are divided into signaling and functional groups [58]. Despite many techniques are available for transcriptome studies, micro-chips developed from quality rice genome sequence were used to identify the regulating reproductive development, hormone signaling and abiotic stress response [59, 60].

Seven-day-old seedlings of Dhagaddeshi (DD) and IR20 cultivars were subjected to drought stress and microarray hybridization of the RNA isolated from samples collected after 3 h and 6 h along with that of control seedlings, was carried out as per manufacturer's instructions (GeneChip® 3' IVT Express Kit User Manual, 2008, Affymetrix). The number of probe sets expressing differentially after 3 h stress is almost double for DD (10,901) than IR20 (5,502) in comparison with the control. However, this difference was less after 6 h with 8,601 for IR20 and 11,041 for DD. Despite the initial delay in sensing drought stress by IR20, differences in transcript levels were more or less mitigated at the 6 h time point. Fructose-bisphosphate aldolase (LOC\_Os01g67860), OsVP1 (LOC\_Os01g68370), auxin response factor 2 (LOC\_Os01g70270) showed high expression levels along with other conserved genes and those of unknown function in DD. Drought stress is known to induce accumulation of osmolytes like proline, glycinebetaine that help in the prevention of dehydration in plants. A significant increase in the accumulation of free proline was observed in both cultivars as the stress duration progressed [61]. The gene expression analysis of DRO1 gene elucidates structural variation and this information is very crucial for breeding rice for drought tolerance in future [52].

#### 6. Drought tolerance varieties

Development of tolerant varieties is the strategy chosen across the field crops including rice. The following are the list of ways by which drought tolerance varieties were developed (**Table 1**). Grain yield was used as a trait to develop drought tolerant varieties and presently, physiological traits are on focus [62]. Most of the characters are influenced by numerous loci termed as Quantitative trait loci (QTL) and they have only minor influence on the trait [63–65]. Marker assisted selection (MAS) is the integration of molecular genetics with artificial selection.

Conventional breeding involves the art of hybrid cross to develop new and improved cultivars. It includes the identification of drought tolerant genetic variants followed by introduction of these traits into popular varieties [66]. It was accepted among the breeders that the existence of drought tolerance variation in the germplasm indicates the presence of stress tolerance genes [67].

However, in conventional breeding programs, only a few parents are involved and their use efficiency in rice accessions remains low because of the inefficient cross-pedigree breeding method, resulting in a narrow genetic base of the developed cultivars [68]. In addition, 15 drought tolerant rice landraces were identified with stable yield under the drought stress while screening both under net house and laboratory evaluation [69].

S. No.	Varieties	Key trait	Country
1	Shabhagidhan	Possess qDTY12.1	India
2	Birsa Vikas Dhan 111	Root QTL from Azucena	India
3	DRR Dhan 42	IR 64 NIL with qDTY4.1 & qDTY2.2	India
4	DRR Dhan 50	Samba Mahsuri sub 1 with qDTY 2.1 & 3.1	India
5	CR Dhan 801	Swarna sub 1 with qDTY 2.1 & 3.1	India

**Table 1.**List of drought tolerant varieties released with drought QTLs through Marker assisted breeding.

#### 7. Recurrent selection (RS)

It involves multiple parents which is an ideal breeding approach to steadily improve the level of quantitative traits in a breeding population. RS was first applied in cross-pollinated crops, maize [70]. In rice, Virmani et al. [71] showed random mating composite population facilitated by IR36ms having recessive genic male sterility for the improvement of restorers and maintainers. However, these two methods were cumbersome and inefficient. In 2001, a mutant of "Sanming Dominant Genic Male Sterile Rice" was found from an F2 population of a cross between SE21S and Basmati370 [72]. The male sterility of this mutant was controlled by a dominant gene and it was fine mapped on chromosome 8 [73]. Further, by multiple backcrosses, they introduced this dominant male sterile (DMS) allele into the genetic background of rice cultivar Jiafuzhan (known as Jiabuyu), which was used to develop 12 drought-tolerant lines through RS [74].

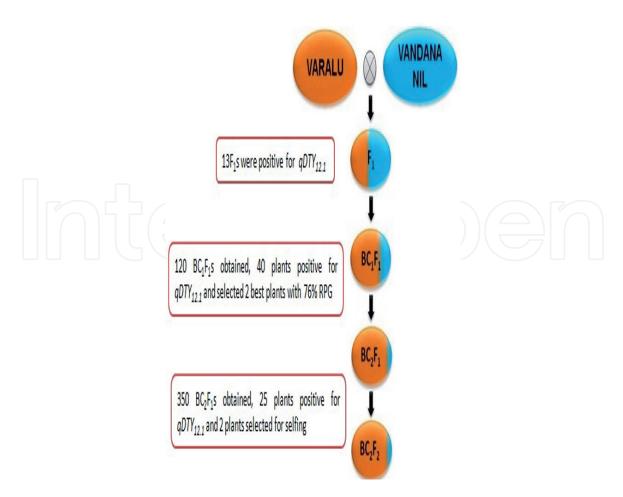
#### 8. Marker assisted back crossing

In backcrossing a donor and recurrent parents are used. Donor parent contains the gene or QTL of interest and the recurrent parent is mega variety or line that is improved by adding the gene or QTL of interest. The donor parent is crossed to the recurrent parent. The progeny of this cross is then crossed back to the recurrent parent (back cross). The progeny of this cross is selected for the added trait and

S.No.	QTL	Chr	Parentage	Reference	
1	1 QTL		CO39 × Moroberekan	Lilley et al. [75]	
2	39 QTLs		CO39 × Moroberekan	Ray et al. [76]	
3	39 QTLs	-	IR64 × Azucena	Yadav et al. [77]	
4	18 QTLs		Azucena × Bala	Price et al. [78]	
5	17 QTLs		Bala × Azucena	Price et al. [78]	
6	28 QTLs		IR58821 × IR52561	Ali et al. [79]	
7	QCMS1.1	1	IR62266 × CT9993	Tripathy et al. [80]	
8	QCMS3.1	3	IR62266 × CT9993	Tripathy et al. [80]	
9	QCMS7.1	7	IR62266 × CT9993	Tripathy et al. [80]	
10	QCMS8.1	8	IR62266 × CT9993	Tripathy et al. [80]	
11	QCMS8.2	8	IR62266 × CT9993	Tripathy et al. [80]	
12	QCMS9.1	9	IR62266 × CT9993	Tripathy et al. [80]	
13	QCMS9.2	9	IR62266 × CT9993	Tripathy et al. [80]	

S.No.	QTL	Chr	Parentage	Reference
14	QCMS11.1	11	IR62266 × CT9993	Tripathy et al. [80]
15	QCMS12.1	12	IR62266 × CT9993	Tripathy et al. [80]
16	15 QTLs		IR64 × Azucena	Hemamalini et al. [81]
17	5 QTLs		CT9993 × IR62266	Zhang et al. [82]
18	23 QTLs		IR1552 × Azucena	Zheng et al. [83]
19	qgy3.1	3	CT9993-5-10-1-M × IR62266-42-6-2	Lanceras et al. [84]
20	qgy4.3	4	CT9993-5-10-1-M × IR62266-42-6-2	Lanceras et al. [84]
21	qGY-2b	2	Zhenshan 97B × IRAT109	Zou et al. [85]
22	qDTY1.1	1	CT9993-5-10-1-M × IR62266-42-6-2	Kumar et al. [86]
23	qDTY12.1	12	Way Rarem × Vandana	Bernier et al. [87]
24	qGy10	10	Tequing × Lemont	Zhao et al. [88]
25	13 QTLs	-	Azucena × Bala	Khowaja, F. S., & Price, A. F [89]
26	7 QTLs	-	Indica × Azucena	Zheng et al. [90]
27	1 QTL		Apo/2 × Swarna	Venuprasad et al. [91]
28	QDS_9.1	9	IR64 × IR77298-5-6-B- 18(Aday Sel)	BP et al. [92]
29	1 QTL		R77298 × Sabitri	Yadav et al. [93]
30	qDTY 3.4	3	Danteshwari × Dagaddeshi	Verma et al. [94]
31	qPN-6-2	6	Xiaobaijingzi × Kongyu131	Xing et al. [95]
32	qDTY3.1	3	TDK1 × IR55419-04	Dixit et al. [96]
33	qDTY6.1	6	TDK1 × IR55419-04	Dixit et al. [96]
34	qPSS8.1	8	IR64 × IRAT177	Trijatmiko et al. [97]
35	qGPP8.2	8	IR64 × IRAT177	Trijatmiko et al. [97]
36	1 QTL		IR64 × Cabacu	Trijatmiko et al. [97]
37	QSnp1b		Teqing × Lemont	Wang et al. [98]
38	QSnp3a		Teqing × Lemont	Wang et al. [98]
39	QSnp11	7	Teqing × Lemont	Wang et al. [98]
40	QGyp2a		Teqing × Lemont	Wang et al. [98]
41	QSf8		Teqing × Lemont	Wang et al. [98]
42	qDTY3.2		Swarna × WAB	Saikumar et al. [99]
43	qPDL1.2	1	Appo × Moroberekan	Sellamuthu et al. [100]
44	qHI3	3	Appo × Moroberekan	Sellamuthu et al. [100]
45	qDTY2-2	2	MRQ74 cultivar	Shamsudin et al. [101]
46	qDTY3-1	3	MRQ74 cultivar	Shamsudin et al. [101]
47	qDTY1-3	1	Dular × IR62-21	Catolos et al. [102]
48	qDTY8-1	8	Dular × IR62-21	Catolos et al. [102]

**Table 2.**List of QTLs reported for drought tolerance associated traits in rice.



**Figure 1.**Back crossing flow chart to introduce drought QTL) (adopted from Balija et al. [103].

again subjected to back cross with the recurrent parent. This process is repeated to obtain a line as identical as possible to the recurrent parent with the addition of the gene of interest that has been added through breeding.

Among the QTLs for drought stress tolerance (**Table 2**), qDTY12.1 offers significant yield under reproductive-stage drought stress by contributing 51% genetic variance [87] and is available in Vandana NIL (near isogenic line). Hence, this QTL was introduced into Varalu (WGL 14377× CR-544-1-2) which is a popular variety cultivated in upland areas of India by back crossing method (**Figure 1**) [103].

Later, responsible genomic regions have been identified and are popularly known as molecular markers. They were used to develop drought tolerant varieties by a process known as marker assisted selection or breeding [66].

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