

1 **Probable omnigenic effect and evolutionary insights of aerobic adaptation allele**
2 **OsNCED2^T on qDTY12.1 effecting grain yield under reproductive stage drought stress in**
3 **rice**

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6 **Short Title**

7 Omnigenic effect of qDTY12.1 and its evolution in rice varieties

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20 **Author's contribution**

21 PC and AN conceptualized the idea, JLK, BB, KJ retrieved the sequence information from
22 different databases, PC, DBN, AK, NB performed the analysis, BS, PC, CB, SS wrote the
23 manuscript, and SS coordinated the entire study

24

25 **Competing interests**

26 Authors declare no competing interests

27 **Abstract**

28 Yield associated quantitative trait loci (qDTY) under drought stress provides significant
29 advantage for grain yield in rice. The major, stable qDTY12.1 was identified in a mapping
30 population developed from upland cultivars Vandana and Way Rarem. Further, introgression line
31 comprising of qDTY12.1 genomic region was characterized to have multiple genes (NAM,
32 DECUSSATE) regulating the drought tolerance under severe drought stress substantiated
33 through recently proposed omnigenic model for complex traits. Recently, plastid localized
34 NCED2^T allele present within the qDTY12.1 genomic region was characterized for conferring
35 aerobic adaptation in lowland varieties. Since, NCED2^T is evolutionary fixed in upland cultivars
36 and Vandana was found to have the favorable allele of NCED2^T, we hypothesized that this
37 favorable allele might confer omnigenic effect on qDTY12.1 genes. Our evolutionary analysis
38 using non synonymous SNPs present in genes namely NCED, NAM, and DECUSSATE and
39 qDTY12.1 genomic regions showed specific grouping of Vandana with upland cultivars only for
40 NCED gene and its adjoining genomic regions. However, non synonymous SNPs in NAM and
41 DECUSSATE genes and its adjoining genomic regions of drought tolerant varieties were closely
42 related and grouped together in the phylogenetic analysis. Moreover, ecotype specific
43 differentiation and greater nucleotide difference with wild relatives was also observed for
44 DECUSSATE gene in rice. This finding indicates differential evolution of qDTY12.1 regions for
45 upland and drought tolerance and omnigenic effect of NCED2^T gene in qDTY12.1. Further, we
46 propose a breeding model for enhancing genetic gain for yield under severe drought stress by
47 incorporation of NCED^T, qDTY12.1 and other drought tolerant QTLs for membrane stability in
48 rice.

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55 Introduction

56 Abscisic acid (ABA) is a major plant hormone which plays a significant role in drought
57 tolerance in plants (Zhang et al, 2006). Plants adaptation to water limitation conditions are tightly
58 regulated through abscisic acid biosynthesis (Xiong and Zhu, 2003). The plastid localized NCED
59 genes (9-*cis*-epoxy-carotenoid dioxygenase) are involved in biosynthesis of precursors required
60 for the synthesis of ABA in plants (Vallabhaneni et al, 2010). In rice, favorable allele of
61 NCED2^T was specifically fixed in upland cultivars and reported to increase the cellular ABA
62 levels and lateral roots (Lyu et al, 2013). Recently, NCED2^T allele was functionally
63 characterized for the aerobic adaptation in upland cultivars (Hu et al, 2020). This gene is located
64 in the chromosome number 12 of rice at 14, 232,905 to 14,234,854 Mbp.

65 Genetic studies on drought tolerance for yield in rice resulted in the identification of
66 several yield QTLs (qDTY) which relatively maintains yield under drought stress conditions
67 (Kumar et al, 2014). A stable, major, large effect QTL for drought tolerance namely qDTY12.1
68 enhanced drought tolerance of lowland cultivars (Mishra et al, 2013). This QTL was identified
69 and fine mapped using Vandana and Way Rarem mapping population and located within the
70 RM28048 (14,106,460) and RM28166 (17,607,668) markers located in chromosome 12.
71 Besides, peak marker (RM512) was found to be present at 17,395,485 bp in chromosome 12 of
72 this QTL (Dixit et al, 2012). Further characterization of qDTY12.1 showed multiple genes within
73 the QTL region might be regulating the drought tolerance response for yield in rice (Dixit et al,
74 2015). Specifically, two genes namely *OsNAM*_{12.1}: 17,391,342 to 17,392,627 (Dixit et al, 2015)
75 and *OsDEC*: 16,500,219 to 16,504,887 were characterized through multiple approaches as casual
76 genes providing ‘omnigenic effect’ for drought tolerance in the qDTY12.1 (de los Reyes et al,
77 2021).

78 Furthermore, introgression line used in the fine mapping and characterization of
79 qDTY12.1 spanned between RM28048 and RM28166 of qDTY12.1. In addition, both the
80 parents namely Vandana and Way Rarem were upland cultivars. Besides, our preliminary
81 observation using SNP seek database also showed presence of OsNCED2^T allele in Vandana
82 cultivar. Therefore, we hypothesized that both the parents might have OsNCED2^T allele and this
83 favorable allele responsible for efficient ABA synthesis might provide ‘omnigenic effect’ to the
84 causal genes present in qDTY12.1.

85 Methodology

86 SNP seek database of rice was used to retrieve the non synonymous single nucleotide
87 polymorphisms in the selected genes and within qDTY12.1 (Alexandrov et al, 2015).
88 Phyogeny.fr online tool was used for construction of phylogenetic analysis using default
89 parameters (Dereeper et al, 2008). TASUKE tool was used for retrieval of gene sequence from
90 wild relatives of rice (Kumagai et al, 2013). MEGAX software was used for the multiple
91 alignment and neighbor-joining phylogenetic analysis of retrieved sequences (Kuamr et al,
92 2018). Flapjack tool was used for the graphical representation of the non synonymous SNPs in
93 the genes (Milne et al, 2010)

94 Results

95 The presence of OsNCED2^T allele (LOC_Os12g24800) in 3k rice panel was initially
96 analyzed in SNP seek database of rice. The allelic position (14233796) corresponding to the
97 functional alternate alleles (C/T) in the NCED2 gene showed approximately 9% (268 nos) of rice
98 genotypes in 3k panel is having the favorable OsNCED2^T allele. Besides, heterozygous alleles
99 were found in 75 rice genotypes. Further, *indica 3*, *indx*, and *sub tropical japonica* has more
100 number of genotypes containing favorable allele as compared to other ecotypes (Fig.1). Apart
101 from the functional allele, all non-synonymous SNPs identified against Nipponbare reference of
102 the three selected genes (NCED, NAM, DEC) were also analyzed. These three genes were
103 previously characterized for drought tolerance and present within qDTY12.1. Non synonymous
104 SNPs for the three genes were retrieved from seven selected genotypes namely N22 (drought
105 tolerant landrace), Vandana (upland variety), IRAT 104 (upland variety), Swarna, Bala, and
106 FL478 from SNP seek database. The analysis identified 14, 3, and 22 SNPs in NCED, NAM,
107 DEC genes, respectively. Further, phylogenetic reconstruction of selected genotypes was
108 performed using the non-synonymous SNPs identified in the three genes (Fig. 2).

109 The phylogeny for NCED gene showed seven genotypes were separated into two clusters
110 and Nipponbare was present as outgroup. The first cluster consists of drought tolerant and upland
111 varieties namely N22, Vandana, and IRAT 10. The remaining susceptible genotypes were
112 present in cluster II of the phylogeny. Similarly, phylogeny of NAM gene divided seven
113 genotypes into two clusters. The upland or drought tolerant cultivar namely N22, IRAT 104 were
114 present in one group along with Swarna and Nipponbare. However, Vandana, Bala, and FL478

115 were present in cluster II. Also, N22 and IRAT 104 were present in same cluster for DEC gene in
116 the phylogeny. Thus, initial phylogenetic analysis for the functional genes clearly showed
117 drought tolerant/upland cultivar, N22 and IRAT 104 were always grouped in same cluster for
118 three genes. In contrast, Vandana was grouped along with N22 and IRAT 104 only for NCED
119 gene and were grouped together with susceptible cultivars for remaining two genes.

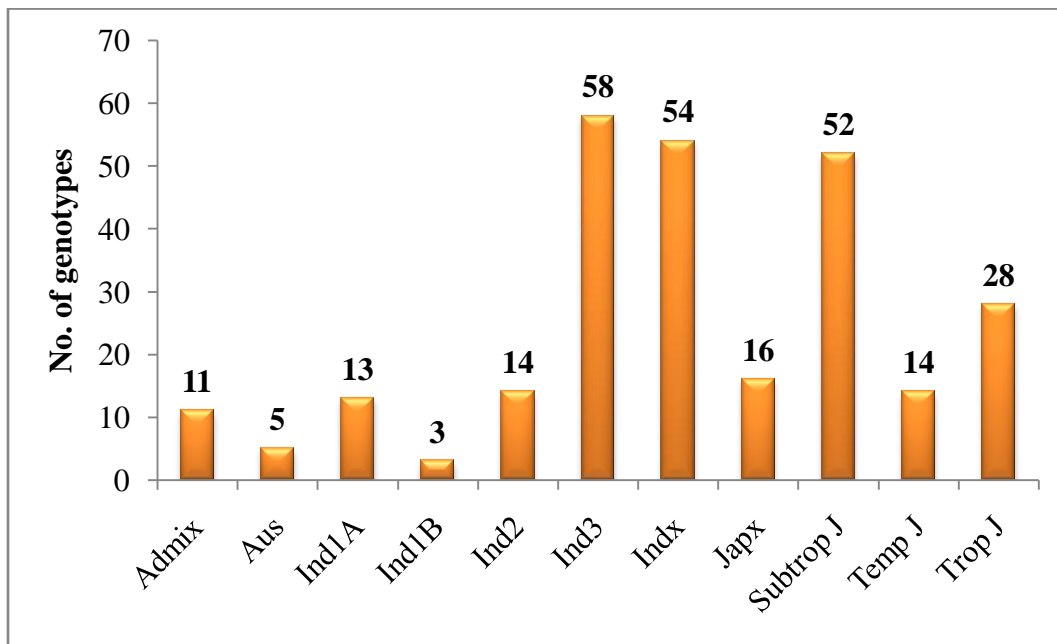
120 We hypothesized that differential evolution of genomic regions within the qDTY12.1
121 might be the reason for the grouping of upland genotype Vandana with other genotypes namely
122 N22 and IRAT 104. Hence, phylogenetic reconstruction was again performed using all the non
123 synonymous SNPs spanning the qDTY12.1 for the seven genotypes. The qDTY12.1 (14-18
124 Mbp) in chromosome 12 were divided into four different genomic blocks (14-15 Mbp, 15-16
125 Mbp, 16-17 Mbp, 17-18 Mbp) and analyzed for the grouping of selected genotypes. The analysis
126 showed there were 681, 322, 306, and 180 non synonymous SNPs within the four genomic
127 blocks, respectively. Further, Vandana, N22, and IRAT 104 were grouped together only in 14-15
128 Mbp genomic region of qDTY12.1. But, Vandana was grouped along with the susceptible
129 genotypes and N22 and IRAT 104 were present in same cluster for the 15-16 Mbp genomic
130 region of qDTY12.1. These findings showed not only the selected genes but there was also
131 differential evolution of genomic regions within qDTY12.1. Accordingly, we have predicted the
132 genomic regions for the genotypes N22, Vandana, IRAT 104, and Way Rarem (Fig. 3f). The
133 entire qDTY12.1 was divided into two genomic segments or blocks. The first segment comprises
134 of favorable allele of NCED2^T present only in upland genotypes namely Vandana, IRAT 104,
135 and Way Rarem. The second block comprises of genomic regions specifically in N22, IRAT 104
136 and Way Rarem. Further, first and second genomic block in qDTY12.1 provides early drought
137 response and yield maintenance under drought, respectively. Thus, we assume that NCED2^T
138 allele might have an omnigenic effect on the genomic blocks of qDTY12.1.

139 The presence of the favorable allele in OsNCED2^T was also analyzed in wild relatives of
140 rice. The analysis showed only one accession of *Oryza rufipogon* (W1807) has the favorable
141 allele for NCED2 gene (Fig. 4). Then, non synonymous substitutions in the DECUSSATE gene
142 were also analyzed in all rice genotypes of the 3k panel and wild relatives. The haplotype blocks
143 was constructed in different rice ecotypes for the DECUSSATE gene and analyzed for the
144 specific fixation of non reference alleles (Fig. 5). The analysis showed sub tropical and temperate
145 japonica retained the reference alleles of Nipponbare. However, all other ecotypes including

146 tropical japonica showed non synonymous substitutions in the gene of varying proportions.
147 Additionally, many wild relatives also showed non synonymous substitutions in the coding
148 regions of the gene (Fig. 6). Thus, DECUSSATE gene and the genomic regions of the qDTY12.1
149 might have evolutionary significance in rice.

150 **Fig. 1.** Distribution of favorable allele (OsNCED2^T) in different rice accessions in SNP seek
151 database

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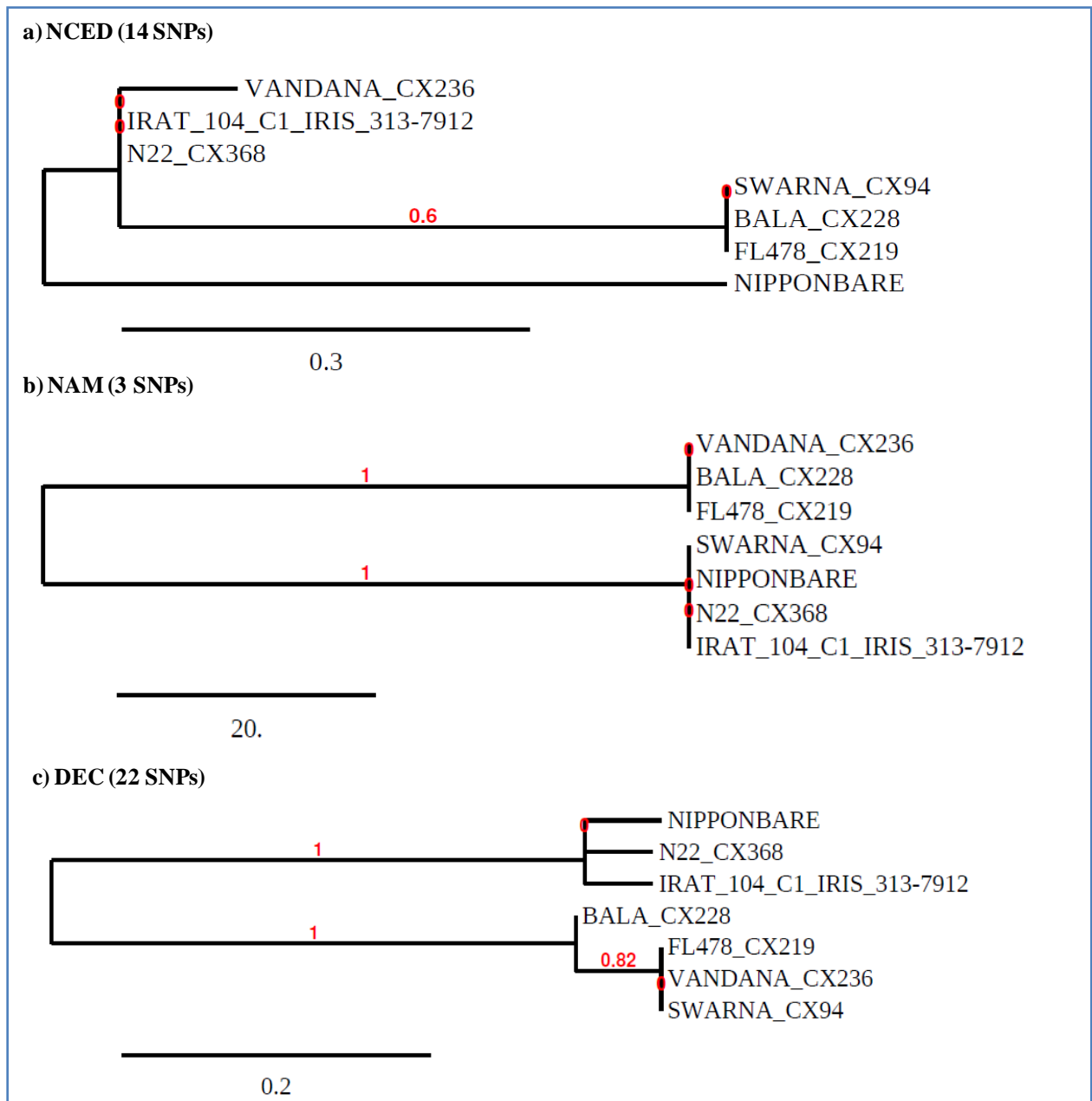
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161 **Fig. 2.** Phylogenetic analysis of seven genotypes using SNPs present in three selected genes

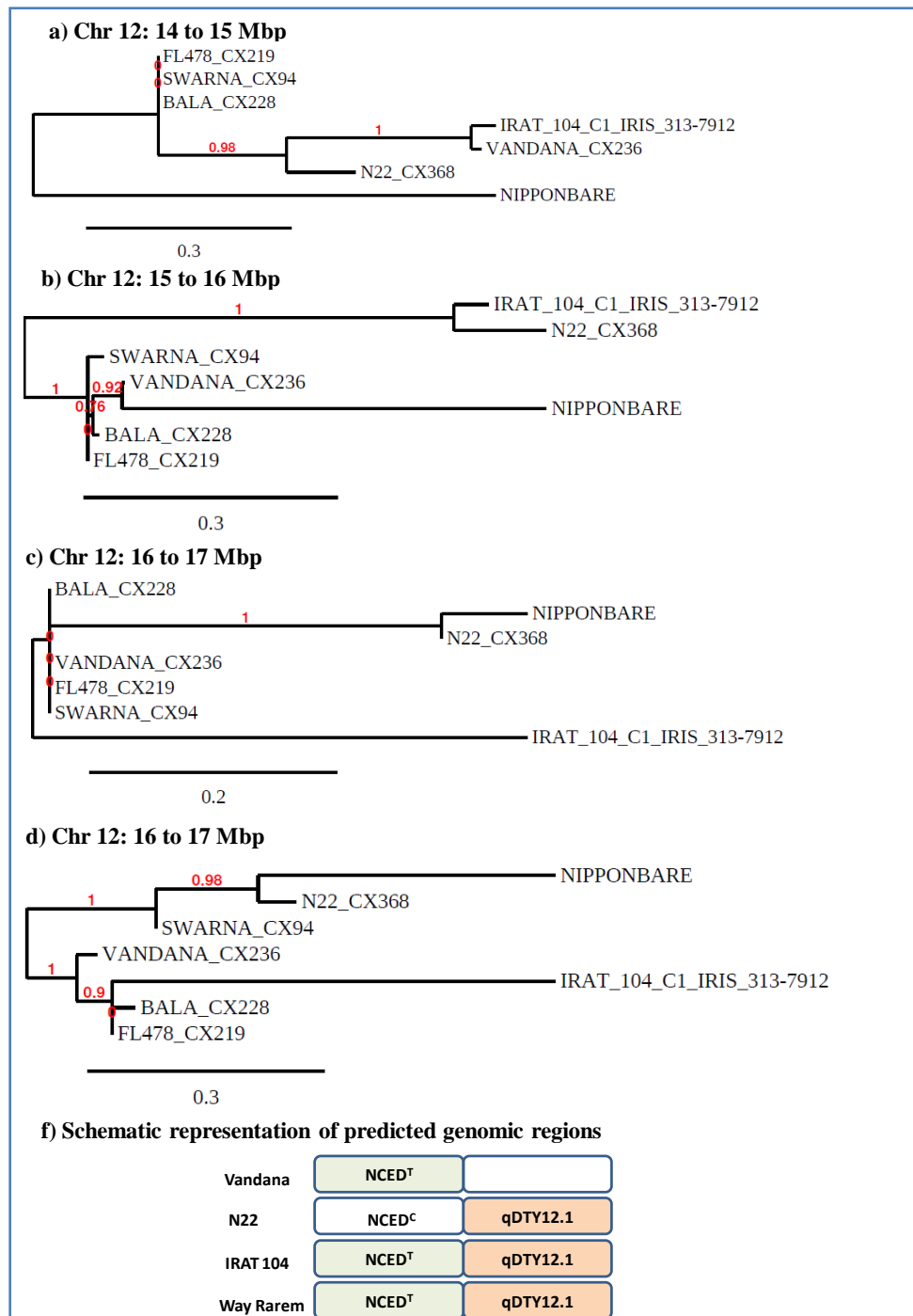


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163 **Fig. 2** Phylogenetic analysis of seven genotypes using SNPs present in three selected genes. a-
164 neighbor-joining phylogeny using non synonymous SNPs in NCED gene, b- neighbor-joining
165 phylogeny using non synonymous SNPs in NAM gene, c- neighbor-joining phylogeny using non
166 synonymous SNPs in DECUSSATE gene. Numbers in bracket indicates total non synonymous
167 SNPs identified in the three selected genes.

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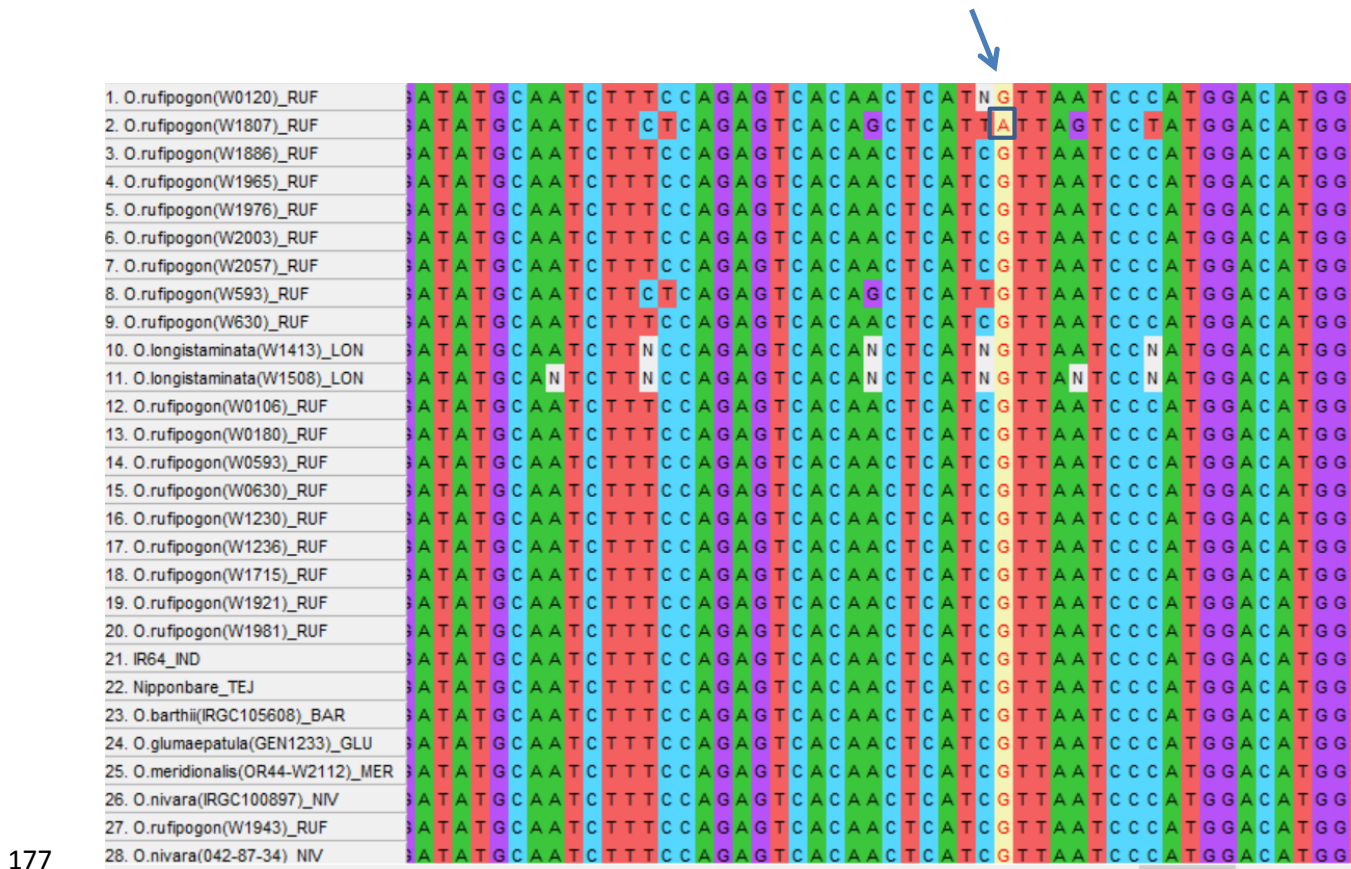
169 **Fig. 3.** Phylogenetic analysis of seven genotypes using SNPs identified in qDTY12.1



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171 **Fig. 3.** Phylogenetic analysis of seven genotypes using SNPs identified in qDTY12.1. a,b,c,d
 172 represents neighbor-joining phylogenetic tree for different genomic regions. f- represents two
 173 genomic blocks in qDTY12.1 and predicted genotypes for four cultivars. Green color fill
 174 indicates presence of favorable allele for NCED gene, brown color fill indicates favorable alleles
 175 for qDTY 12.1

176 **Fig. 4.** Multiple alignments of NCED gene in rice wild relatives.



178 **Fig. 4.** Multiple alignment of NCED gene in rice wild relatives. Arrow indicates the alignment
179 position and boxed nucleotide indicate the favorable allele in one of the wild relatives (*Oryza*
180 *rufipogon*).

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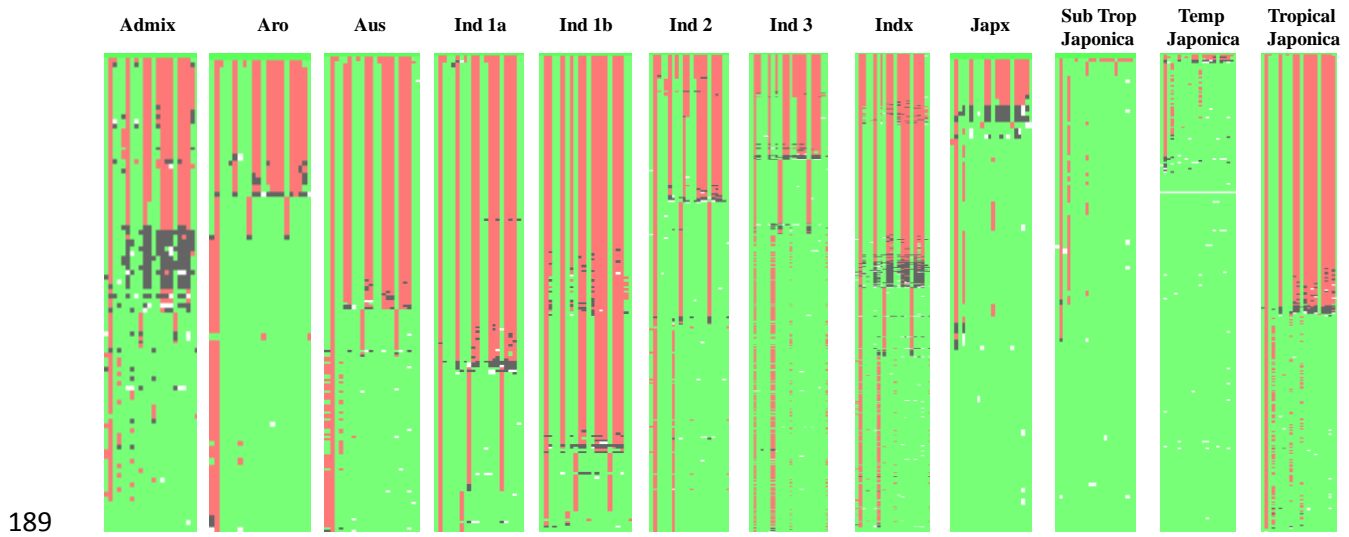
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188 **Fig. 5.** Graphical representation of haplotypes of DECUSSATE gene in different ecotypes of rice



190 **Fig. 5.** Graphical representation of haplotypes of DECUSSATE gene in different ecotypes of rice
191 Green, red and black represents similar, alternate and heterozygous allele to Nipponbare
192 reference gene sequence. Non availability of sequence information was indicated in white color
193 boxes.

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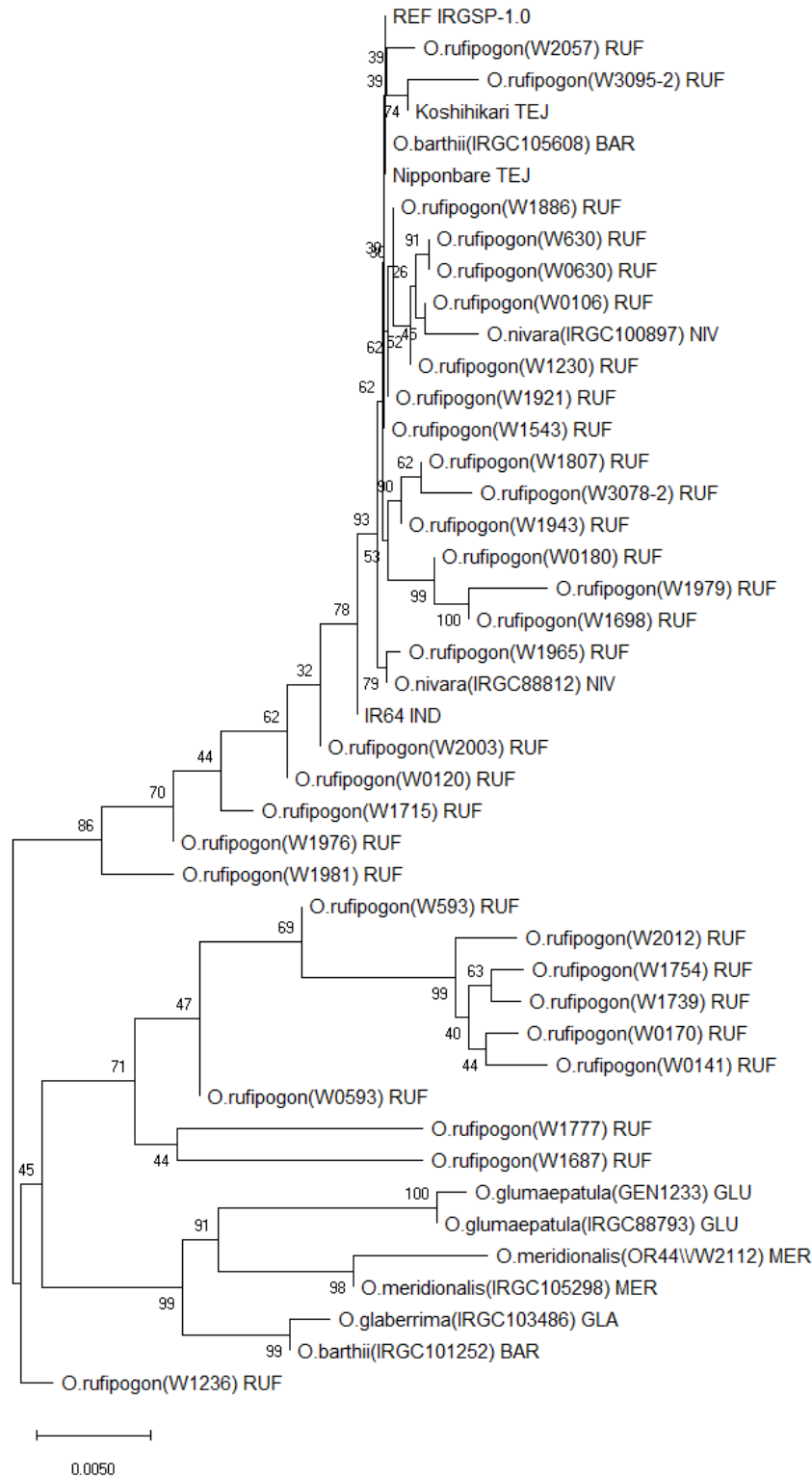
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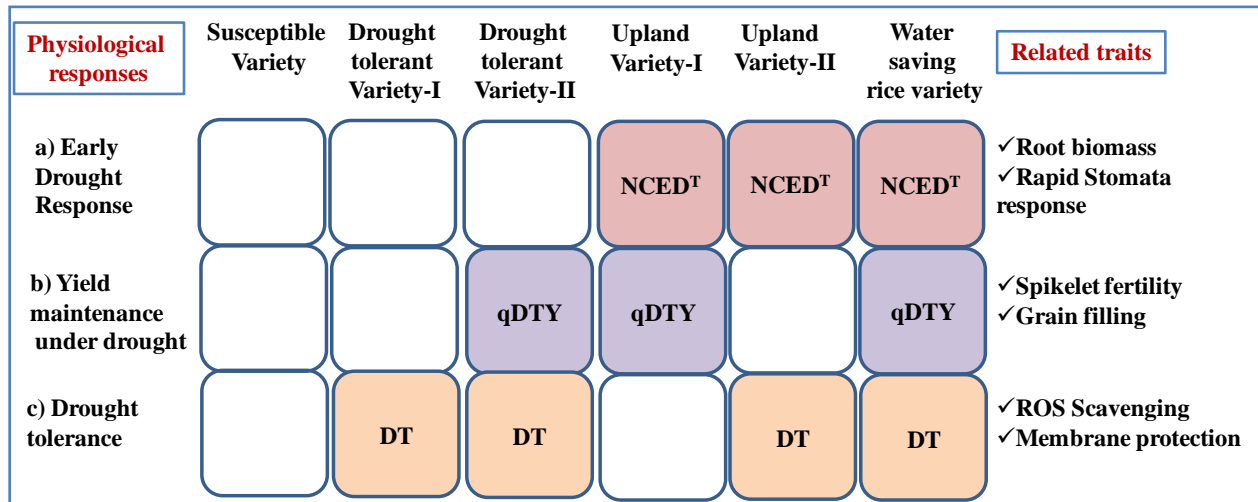
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204 **Fig. 6.** Phylogenetic analysis of DECUSSATE gene in wild relatives of rice



206 **Fig.7.** Schematic representation for enhancing the genetic gain for yield under severe drought
 207 stress in rice for the development of water saving rice varieties



208

209 **Fig.7.** Schematic representation for enhancing genetic gain for yield under severe drought
 210 stress in rice. Breeding models for the development of water saving rice varieties in the present model
 211 comprises of combination of early drought response (pink color), yield maintenance (purple
 212 color) and drought tolerance (brown color) conferred by NCED^T, qDTY12.1, and drought
 213 tolerant QTLs. White color indicates absence of above mentioned traits in susceptible varieties.
 214 Water saving varieties comprises of all three physiological responses under severe drought stress
 215 conditions. Upland or drought tolerant varieties comprises of any two of above mentioned
 216 physiological responses. The related traits for these three physiological responses are given in
 217 right side of the schematic diagram.

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225 Discussion

226 The first findings of this study are qDTY12.1 region (14-18Mbp) in rice has evolved
227 differently and contributed to both upland adaptation and drought tolerance in rice. The
228 differential grouping of Vandana (upland cultivar but drought susceptible at reproductive stage)
229 and N22 (drought tolerant) in phylogeny for different genomic regions within qDTY12.1,
230 differential fixation of DECUSSATE alleles in rice ecotypes, and diversity of OsDEC gene
231 sequences in wild rice relatives substantiates the evolutionary significance of genomic blocks
232 within qDTY12.1. In agreement with our findings, OsNCED2^T allele was found only in upland
233 cultivars (Lyu et al, 2013) and enhanced ABA levels and lateral root density contributing to
234 aerobic adaptation of rice (Hu et al, 2020). Additionally, a comprehensive GWAS analysis using
235 3.3 million SNPs also identified several significant root length associated SNPs in the entire 12th
236 chromosome specifically in indica than japonica ecotypes (Zhao et al, 2018). In rice, 11th and
237 12th chromosome was found to be evolved and associated with richness of disease resistance R
238 genes (Rice chromosome sequencing consortium, 2005). Similarly, we presume that the 12th
239 chromosome may also be differentially evolved and associated for ecotypes diversification and
240 drought tolerance in rice. However, rather than gene duplications which are responsible for
241 multiple homologs of R genes in 11 and 12th chromosome, evolutionary mechanisms might be
242 different in 12th chromosome especially within qDTY12.1 genomic regions which are associated
243 with combinations of drought tolerance, root biomass and aerobic adaptations.

244 The second findings from this study is the possible ‘omnigenic effect’ of OsNCED2^T
245 allele with qDTY12.1 in rice. The omnigenic model was initially proposed for disease related
246 traits in humans wherein strong trait associated loci along with adjoining genes and other genes
247 spread in the genome are interconnected and regulates the phenotype of complex traits (Boyle et
248 al, 2017). Recently, de los Reyes (2021) identified DECUSSATE gene in rice as one of the
249 causal gene in qDTY12.1 which regulates panicle development under drought stress.
250 Additionally, another causal gene OsNAM_{12.1} and several other candidate genes have been
251 reported for controlling the drought tolerance mechanism in qDTY12.1 (Dixit et al, 2015). All
252 these reports strengthen the omnigenic effect of genes in qDTY12.1 for conferring yield
253 advantage under drought stress. Since, both the OsNCED2^T (Hu et al, 2020) and OsNAM_{12.1}
254 (Dixit et al, 2015) regulates root length and grain yield, Vandana cultivar consist of favorable
255 allele of NCED2 gene, and it is located within the genomic regions studied for the

256 characterization of qDTY12.1, we propose that OsNCED^T gene functions as omnigenic gene and
257 regulates the qDTY12.1 for the maintenance of yield under severe drought stress. The enhanced
258 ABA levels and signaling due to OsNCED^T allele might be considered as omnigenic mechanism
259 for the qDTY12.1 conferring drought tolerance. However, this assumption requires further
260 studies and characterization.

261 The development of water saving rice varieties has the potential to ensure sustainable rice
262 production in coming decades (Luo et al, 2010). In this regard, qDTY12.1 which showed
263 consistent yield performance and contributed to 23% phenotypic variance for yield under severe
264 drought stress conditions is highly essential for improvement of rice varieties (Mishra et al,
265 2013). Additionally, membrane stability QTLs has also been identified in rice for drought
266 tolerance (Tripathy et al, 2000; Shanmugavadivel et al, 2019). Thus, a breeding model through
267 introgression of NCED2^T, qDTY12.1, and QTLs/genes conferring membrane stability for
268 drought stress has been proposed for further enhancement of genetic gain for yield under severe
269 drought stress in rice (Fig. 7). The proposed model incorporates the physiological processes
270 namely rapid response, yield maintenance, and membrane stability under severe drought stress
271 conditions.

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