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ORIGINAL RESEARCH

DECUSSATE network with flowering genes explains the variable effects of *qDTY12.1* to rice yield under drought across genetic backgrounds

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Abstract

The impact of *qDTY12.1* in maintaining yield under drought has not been consistent across genetic backgrounds. We hypothesized that synergism or antagonism with additive-effect peripheral genes across the background genome either enhances or undermines its full potential. By modeling the transcriptional networks across sibling *qDTY12.1*-introgression lines with contrasting yield under drought (LPB = low-yield penalty; HPB = high-yield penalty), the *qDTY12.1*-encoded *DECUSSATE* gene (*OsDEC*) was revealed as the core of a synergy with other genes in the genetic background. *OsDEC* is expressed in flag leaves and induced by progressive drought at booting stage in LPB but not in HPB. The unique *OsDEC* signature in LPB is coordinated with 35 upstream and downstream peripheral genes involved in floral development through the cytokinin signaling pathway. Results support the differential network rewiring effects through genetic coupling–uncoupling between *qDTY12.1* and other upstream and downstream peripheral genes across the distinct genetic backgrounds of LPB and HPB. The functional *DEC*-network in LPB defines a mechanism for early flowering as a means for avoiding the drought-induced depletion of photosynthate needed for reproductive growth. Its impact is likely through the timely establishment of stronger source-sink dynamics that sustains a robust reproductive transition under drought.

1 | INTRODUCTION

The Green Revolution created modern high-yielding cultivars of rice (*Oryza sativa* L.) with morphological and physiological attributes optimal for environments with ample water and nutrients (Khush, 1995, 2001, 2005, 2013a, 1995b; Peng et al., 2008). However, the increased incidence of erratic rainfall patterns, diminishing water resources, and depletion of arable

Abbreviations: ABA, abscisic acid; *DEC*, *Decussate*; FPKM, fragments per kilobase of transcript per million; GO, gene ontology; HPB, high-yield penalty backcross introgression line; IRRI, International Rice Research Institute; LPB, low-yield penalty backcross introgression line; NPF, negative propensity fraction; *OsDEC*, *DECUSSATE* gene; PPF, positive propensity fraction; PS, propensity score; *qDTY12.1*, yield under drought QTL on chromosome-12; WR, Way Rarem; ZEP, zeaxanthin epoxidase.

lands paints the new reality at which crop production must be undertaken to ensure yield stability under increasing global food demands and steadily rising population. With this reality in mind, innovative and holistic paradigms in plant breeding become critical to the development of the next generation of crop cultivars that can absorb this conglomeration of ecological factors while minimizing penalties to yield. The creation of novel ideotypes with resilience to drought-prone environments, for example, holds great promise for maximizing yield under suboptimal conditions (de los Reyes, 2019; Pabuayon et al., 2020).

With the reported drought-related yield losses in rice ranging from 18 to 97% (Sandhu & Kumar, 2017), robust approaches in breeding, like quantitative trait loci (QTL) introgression and pyramiding, for example, become the most vital components of a holistic strategy for addressing the needs of subsistence rice farmers in regions that are highly prone to either periodic episodes of drought or persistent drought (Dar et al., 2020; Lei et al., 2016; Lottering et al., 2020; Serraj et al., 2011). The discovery and subsequent pyramiding of large-effect QTLs that function at the reproductive stage (i.e., *qDTYs* for yield maintenance under drought) (Supplemental Table S1), have led to incremental but major improvements in the yield potential of widely grown rice cultivars that regularly incur significant penalties due to reproductive-stage drought (Singh et al., 2016; Swamy et al., 2014; Vikram et al., 2011). Among the most well-characterized and of highest importance is the yield under drought QTL on chromosome-12 (*qDTY12.1*), because of its more consistent effects in reducing the penalty to yield across growing environments (Bernier et al., 2007, 2009; Mishra et al., 2013). Fine-mapping of *qDTY12.1* in the 'Way Rarem' (WR) × 'Vandana' derived population defined its boundaries within 3.1 cM on the long-arm of chromosome-12, estimated to be 1.554 Mbp with physical coordinates in the Nipponbare *RefSeq* between 15,848,736 bp to 17,401,530 bp (Dixit et al., 2012).

Initial attempts to understand many of the mechanisms by which *qDTY12.1* is able to impart such large positive effects as a yield QTL pointed to a number of candidate genes (Dixit et al., 2015; Swamy et al., 2011; Yadav et al., 2019). However, while these genes provided important advances, many of the mechanisms that have been uncovered so far appeared to be involved in stress avoidance during vegetative growth, without direct link to reproductive growth, source-sink partitioning, and/or grain development, which are more meaningful to yield maintenance (Henry et al., 2014, 2015, 2019; Raorane, Pabuayon, Miro et al., 2015). For instance, a recent study showed the importance of the *qDTY12.1*-encoded $OsNAM_{12.1}$ transcription factor (*Os12g0477400*) in the regulation of root development and architecture as a mechanism of drought avoidance during vegetative growth (Dixit et al., 2015). In addition, a meta-analysis of 53 grain yield-related QTLs identified six loci within the meta-QTL on *qDTY12.1* that are not directly associated with yield processes (Swamy et al., 2011).

Core Ideas

- Optimal effect of *qDTY12.1* on yield maintenance under drought is defined largely by its interaction with the genetic background.
- The *qDTY12.1*-encoded *DECUSSATE* gene (*OsDEC*) is a putative regulator of flowering during drought through cytokinin signaling.
- *OsDEC* is the core of a drought response genetic network at reproductive transition, comprised of flowering-associated genes from across the genome.
- Coupling of *qDTY12.1*-encoded *OsDEC* allele with a battery of compatible peripheral flowering-associated genes across the genome creates an optimal flowering response genetic network during drought.
- Optimal *OsDEC* network facilitates an earlier transition to flowering and efficiently channels photosynthates from the progressively depleting vegetative source to maintain the strength of reproductive sink.

Perhaps the most interesting aspect of *qDTY12.1* was the fact that this locus did not exhibit a positive effect on yield maintenance in its native genetic background (i.e., original donor), which is the Indonesian upland cultivar WR (Bernier et al., 2007). However, significant positive effects of the *qDTY12.1* in minimizing yield penalty under drought were observed in recombinants with the Indian cultivar Vandana, which has drought tolerance at the vegetative stage but with high drought penalty to yield (Bernier et al., 2007; Kumar et al., 2014). These seminal observations inspired the initial hypothesis that the full effects of *qDTY12.1* require some kind of synergy and complementation with other minor peripheral genes in the genetic background that cannot be identified at high statistical confidence by the resolution of QTL mapping (Yadav et al., 2019). Researchers have been trying to identify such network of genes among the *qDTY12.1* genes themselves, but so far no truly significant leads apart from vegetative stage drought avoidance have been uncovered (Henry et al., 2014, 2015, 2019; Raorane, Pabuayon, Varadarajan et al., 2015).

With the observations that some introgression derivatives of *qDTY12.1* exhibited consistent yield retention under drought, while others did not, the question was raised as to why the presence of the *qDTY12.1* allele of WR alone,

as facilitated by marker-assisted selection, would not be sufficient in providing the expected positive effects across different genetic backgrounds or even within similar genetic backgrounds (Yadav et al., 2019). We hypothesized that in specific derivatives carrying the same *qDTY12.1* allele from WR where the expected positive effects were not manifested, genetic recombination may have created some kind of coupling–uncoupling effects involving many other alleles in the genetic background that are peripheral but synergistic to *qDTY12.1* functions, with the *qDTY12.1* genes themselves acting as the core of the mechanism (i.e., epistatic effects or network rewiring effects) (Kitazumi et al., 2018; Pabuayon et al., 2021). This hypothesis built its strength from the recently proposed Omnigenic theory, which postulated that complex traits are controlled by not only a core set of loci with quantifiable effects, but also by a genome-wide cohort of other peripheral loci whose individual effects are minute but additive to either positively or negatively complement the core effects to account for larger proportions of the total phenotypic variance (Boyle et al., 2017).

To dig deeper into the yield-related function of *qDTY12.1* while also addressing the coupling–uncoupling and network rewiring hypotheses, we investigated a minimal comparative panel established at the International Rice Research Institute (IRRI) that models the contrast between positive net gain and negative net gain from *qDTY12.1* effects across potentially contrasting combinations of peripheral alleles in the genetic background (Kumar et al., 2020). This panel is comprised of the cultivar WR, the original donor of *qDTY12.1*, the drought-sensitive mega-cultivar IR64 as recipient of *qDTY12.1*, and two IR64 sibling backcross derivatives with the *qDTY12.1* of WR introgressed through a bridge donor recombinant from Vandana, hence low-yield penalty and high-yield penalty backcross introgression lines (LPB and HPB, respectively) (Supplemental Figure S1) (Sandhu & Kumar, 2017).

A cautionary thinking is that LPB and HPB are considered to have uniform genetic backgrounds but only at the extent and resolution afforded by marker-based genotyping, and not based on whole-genome sequence assembly. That being said, the potential contributions of other hidden introgressions that could possibly be traced across their pedigrees (i.e., either WR or Vandana), beyond what can be ascertained by the resolution of marker-assisted selection of the foreground and background, must not be excluded as potential sources of cryptic variations between LPB and HPB. By in-depth analysis of the drought-response transcriptomes at vegetative, reproductive (booting), and grain-filling stages under field drought, along with the modeling of co-expression networks, we identified the first candidate gene of *qDTY12.1* with a convincing direct link to processes that could modulate the timing of reproductive transition under the limiting source-sink status during drought. We report here the identification of *DECUSSATE* gene *(OsDEC)*, a single copy locus in the rice genome (*Os12g0465700*) and first identified as a regulator of leaf phyllotaxy (Itoh et al., 2012), as a crucial gene of *qDTY12.1* that facilitates efficient panicle development under drought, likely through the cytokinin signal transduction pathway.

2 | MATERIALS AND METHODS

2.1 | Minimal comparative panel

Based on extensive genotyping and yield evaluation under progressive drought, a minimal comparative panel illustrating the differential effects of *qDTY12.1* across genetic backgrounds was established at IRRI (Dixit et al., 2014; Kumar et al., 2008, 2014). This panel was comprised of the Indonesian upland cultivar WR (IRGC122298) as the original donor of *qDTY12.1*, the drought-sensitive mega-cultivar IR64 as the recurrent parent used for backcross introgression of the *qDTY12.1* from WR, and two sibling introgression lines of IR64 carrying the *qDTY12.1* from WR (IR102784:2-42-88-2-1-2, IR102784:2-90-385-1-1-3) designated as LPB and HPB lines, respectively (Kumar et al., 2020; Yadav et al., 2019).

2.2 | Drought experiments and tissue sampling

Parallel replicated experiments across the minimal comparative panel were conducted at IRRI's Ziegler Experiment Station in Los Banos, Laguna, Philippines (14°30′ N, 121°15′ E) during the 2017 wet season (WS; June to November 2017) for the irrigated and drought conditions. The field experiment was an alpha-lattice design with three replicates and three individual plants per replicate that were single-seed transplanted in the field plots after establishing for 21 d in seedling beds. Control plots were maintained in standard irrigated levy based on IRRI's standard protocols, while the drought plots were established inside a rain-out shelter facility for drought screening next to the irrigated plots (Supplemental Figure S2) (Henry et al., 2011, 2014, 2015; Torres & Henry, 2018; Torres et al., 2013; Villa et al., 2012). Both the irrigated and drought plots were given continuous irrigation corresponding to 5 cm of standing water until 30 d after transplanting (DAP) or 51 d after sowing, when progressive drought was initiated for the treatment group by withholding water until the end of the season. A life-saving irrigation was applied to the drought plots at the point when extensive leaf rolling was observed to promote survival until harvest (Torres et al., 2012).

Tissue sampling was conducted on three plants per replicate in both the irrigated and drought conditions. Samples were comprised of pooled flag leaves with the connected leaf

sheath surrounding the developing panicle. The timing of tissue sampling was synchronized as defined by the days counted backward (t_{-1} = vegetative) or forward (t_1 = grain-filling) from the reference time-point (t_0 = booting) to generate developmentally comparable flag leaf transcriptomes across genotypes. At t_{-1}, samples were collected from three plants from each genotype and experimental plot, 7 d after the initiation of progressive drought. At t_0, samples were collected from three plants from each genotype and experimental plot, 12 d prior to panicle extrusion (heading). At t_1, samples were collected 5 d after anthesis when the developing grains had milky consistencies and more than 50% of the plants had completely flowered. All samples were collected at the same time of the day (between 8:00 a.m. and 10:00 a.m.) and were immediately frozen in liquid nitrogen. Panicle length (mm), plant height (cm), tiller number plant^{-1}, reproductive tiller number plant^{-1}, and biomass plant^{-1} were recorded from all experimental plots.

2.3 | Transcriptome analysis by RNA-Seq

Total RNA was extracted from frozen flag leaves using the miRVana™ miRNA isolation kit according to manufacturer's protocol (Invitrogen, Carlsbad, CA). RNA from three individual plants in each genotype were pooled to create a composite sample representing each replicate. Two independent RNA-Seq libraries were constructed from the pooled RNA across genotypes, developmental stages, and treatments, according to standard in-house protocols (Kitazumi et al., 2018). The indexed RNA-Seq libraries were sequenced twice in the Illumina HiSeq3000 (Oklahoma Medical Research Foundation) by strand-specific and paired-end sequencing at 150-bp with 30 to 40 million sequence reads per run.

Raw RNA-Seq data were processed and assembled through the established in-house data analysis pipeline (Kitazumi et al., 2018). Sequence output from the indexed RNA-Seq libraries (NCBI PRJNA717284) was preprocessed with Cutadapt (v2.10) and mapped against the Nipponbare *RefSeq* and corresponding GFF gene models (IRGSP-1.0) using the Tophat2 (v2.1.1) and Bowtie (v2.2.8.0) (Kim & Salzberg, 2011; Martin, 2011; Sakai et al., 2013). Gene models were further refined using Cuffmerge and differential expression was calculated with Cuffdiff on Cufflinks (v.2.2.1) with default parameters (p-value < .05, FDR = 5%) (Trapnell et al., 2010). Expression of 25,786 annotated protein-coding genes were detected across the RNA-Seq data matrices of irrigated vs. drought-stressed plants at vegetative (V7 to V10), early booting (R1 to R2), and grain-filling (R7) stages. Transcript abundance was expressed as fragments per kilobase of transcript per million (FPKM). Biological interrogation was performed in three windows, that is, global transcriptome (n = 25,786), transcription factor genes (n = 1,340), and stress-related genes (n = 2,589 loci). Transcription factors were extracted from Nipponbare *RefSeq* (https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Oryza_sativa_Japonica_Group/101/). Stress-related genes were based on relevant keywords (Supplemental Table S2).

2.4 | Propensity transformation of RNA-Seq data

Direct comparison of FPKM-based expression is less efficient in extracting biologically meaningful expression patterns (fluxes) because of the confounding effects created by the highly disparate nature of inter-genotypic variation and the stochastic nature of gene expression. Meaningful changes in gene expression are also dependent on the molecular interactions of target genes and their activators or repressors (Kærn et al., 2005; Schwabe et al., 2011). Gene flux theory posits that within the natural competition for transcriptional machinery, genes with low transcript abundances are ultra-sensitive to the effects of other genes (De Vos et al., 2011). Critical loci with low FPKM in one genotype are often discarded, making the directional character of expression fluxes difficult to extract. To address these potential limitations in interpreting the significance of intergenotypic differences, we performed an additional normalization by propensity transformation, which uses within genotype and within treatment comparisons of FPKM-based expression for each locus against the summation across all time-points and against the summation of all loci across the entire dataset (Shu et al., 2020). The FPKM values across the entire transcriptome matrix were Propensity-normalized to generate a propensity score (PS) for each gene locus by

$$Pt_i = ln\left(\frac{\frac{T_i}{\sum_{j=t_1}^{t_3} T_{ij}}}{\frac{\sum_{i=1}^{n} T_j}{\sum_{j=t_1}^{t_3}\sum_{i=1}^{n} T_{ij}}}\right)$$

where Pt_i = propensity transformation of FPKM of transcript I; T_i = FPKM of transcript I; n = total number of transcripts (25,786); j = variable that iterates over datasets of t_1 = vegetative, t_2 = booting, t_3 = grain-filling; and $\underline{i}$ = variable that iterates over the total number of transcript-encoding loci (Supplemental Figure S3). Propensity-transformed datasets (global, transcription factor, and stress-related windows) were filtered at a threshold of $-0.3 < PS > 0.3$ to extract the genes with the largest fluxes. The total of 8,215 loci (out of 25,786) from the global dataset were subjected to k-means clustering to further refine the large cohort into 15 subclusters for PS ≥ 0.3, and 10 subclusters for PS ≤ −0.3.

2.5 | Analysis of transcriptome fluxes and directionality

While the standard approach, based on differentially expressed genes, can give useful insights into cellular processes, the underlying concept tends to be simplistic because cellular responses more often than not involve large number of genes (MacNeil & Walhout, 2011). To capture a more biologically relevant view of the drought response transcriptomes across genotypes, PS data were interrogated to uncover differences in fluxes on a locus-by-locus plane. PS facilitated direct comparison of expression fluxes between genotypes by hierarchical clustering across all transcriptome windows.

The directional character of expression fluxes indicates the degree of transcriptional response modulation. An unmitigated or "untamed" response would have an overabundance of positive transcriptional changes, while a modulated or "tamed" response would be characterized by tightly controlled changes in expression. The directional character of expression fluxes was assessed by comparing the fraction of loci with positive PS (PPF [positive propensity fraction]) to the fraction of loci with negative PS (NPF [negative propensity fraction]), and scored as positive skew (upward arrow), negative skew (downward arrow), or neutral (line segment). Positive skew indicates PPF > NPF, whereas negative skew indicates NPF > PPF, and neutral when PPF = NPF. Genes with PS = 0 (5–9% of total) were excluded.

2.6 | Hierarchical clustering and statistical analysis

Hierarchical clustering and other statistical analyses were performed using JMP® (v14.0.0. SAS Institute Inc.). Mean comparisons of agronomic measurements in rice and Arabidopsis experiments were performed with Tukey HSD following a significant ANOVA at $p = .05$.

2.7 | RiceFREND and KnetMiner analyses

As a preliminary tool for our investigation, the RiceFREND co-expression database (https://ricefrend.dna.affrc.go.jp/) (Sato et al., 2013) was used for two purposes. First, the analysis was performed to determine what putative alliances would form among the *qDTY12.1* genes themselves and between *qDTY12.1* genes and other genes outside the *qDTY12.1* boundary. Second, from the putative alliances formed by and with *qDTY12.1* genes, relevant biological information could be captured with respect to function. Using the default settings of the multiple gene guide tool, co-expression analysis of the 18 *qDTY12.1* genes was performed. KnetMiner was used to determine the enrichment of biochemical, physiological, and agronomic traits associated with various components of the *DEC*-network (http://knetminer.rothamsted.ac.uk) (Hassani-Pak, 2017). RAP-DB loci for the 36 *DEC*-network genes were used as queries for domains (biological processes) in Knetminer using default parameters (Sakai et al., 2013). Knetminer integrates knowledge in the public domain (e.g., gene function, GWAS, Protein, Phenotype, Pathways) to generate biological function maps.

2.8 | Analysis of *Decussate* (*DEC*) knock-out mutants in Arabidopsis

Orthologs of *OsDEC* in *Arabidopsis thaliana* were determined by phylogenetic analysis as *At3G03460* (3Gm) and *At5G17510* (5Gm) (Itoh et al., 2012) (https://plants.ensembl.org/Arabidopsis_thaliana/Info/Index). Mutants from the Arabidopsis Biological Research Center were identified using the Salk Institute T-DNA Express Gene Mapping Tools (http://signal.salk.edu/cgi-bin/tdnaexpress). Seeds were vernalized in 0.1% (w/v) agarose at 4 ˚C for 7 d and grown for 14 d in peat pellets (Jiffy-7) at constant 22 ˚C with 16 h of light (100 µmol m^{-2} s^{-1}) and 60–70% relative humidity. DNA and RNA extraction and genotyping and quantitative polymerase chain reaction analyses were performed according to standard protocols guided by Minimum Information for Publication of Quantitative Real-Time PCR Experiments standards (Supplemental Table S3).

The agronomic performance of *AtDEC* (*Arabidopsis thaliana Decussate* gene) mutants was investigated in growth chamber drought experiments that mirrored the developmental timing of stress in the rice experiments (Harb & Pereira, 2011). A pilot study established the drought conditions at 30% field capacity, 8 d prior to bolting, 27 ˚C d/22 ˚C night, and 40% relative humidity. Control experiments were performed at 70% field capacity, constant 22 ˚C, and 65–80% relative humidity. Vernalized seeds of Col-0, 3Gm, and 5Gm were germinated in peat pellets (Jiffy-7; 42 mm × 65 mm) and grown in two separate growth chambers at constant 22 ˚C with 16 h of light (100 µmol m^{-2} s^{-1}) and 65–80% relative humidity. Drought experiment was performed by growing the plants for 20 d (Col-0; 3Gm) and 16 d (5Gm), before withholding irrigation. Progressive drought was imposed for 14 d by maintaining 30% field capacity, while the control plants were maintained at 70% field capacity. The peat pellets at 30% field capacity received a daily water input to maintain a weight of 33 g (peat pellet + plant + water input). Control and post drought plants were maintained at 65–70 g. Days-to-bolting, days-to-first bloom, days-to-seed set (first silique), total dry biomass $plant^{-1}$, and total seed yield $plant^{-1}$ were determined under irrigated and drought conditions.

3 | RESULTS

3.1 | Agronomic performance under drought across the comparative panel

The comparative panel was subjected to slow but progressive drought in the rain-sheltered drought facility at IRRI from the mid-vegetative through the grain-filling stage (Supplemental Figure S2) (Torres et al., 2012). Integrative analysis of grain yield data extracted from recently published studies under identical drought experimental conditions at IRRI (Yadav et al., 2019) revealed that LPB suffered 74% yield penalty from drought, whereas HPB, WR, and IR64 suffered higher yield penalties of 97.5, 94.6, and 89.1%, respectively (Figure 1a). Yield data from identical field drought experiments performed at IRRI specifically for this study recapitulated the same trends, with 66.3% penalty for LPB, 87.1% for HPB, and 77.3% for IR64 (Yadav et al., 2019). However, WR had lower penalty (58.3%) than previously observed (Figure 1b). While there were year-to-year variations between wet and dry seasons, it was evident that LPB consistently outperformed the other genotypes.

Days to flowering also varied significantly across the comparative panel (Yadav et al., 2019). The LPB showed a drought-induced delay in flowering of only 8 d compared with HPB, WR, and IR64, with delays of 16, 18, and 10 d, respectively (Figure 1c). These differences suggested that LPB may have established a stronger reproductive sink much earlier than the inferior genotypes, and this may have allowed an escape from the negative impacts of drought to resource allocation during the critical stages of floral organ development. Indeed, trends in five other growth components with direct significance to yield potential showed that LPB was superior regarding drought-induced reduction in the number of reproductive tillers, panicle length, total number of tillers, dry biomass plant^{-1}, and plant height (Figure 1d–h). Taken together, significant differences in grain yield and other agronomic attributes relevant to yield between LPB and HPB, suggest that while the sibling *qDTY12.1* introgression lines may be sharing largely similar genetic backgrounds, their yield potentials under drought were significantly different from each other.

3.2 | Transcriptome fluxes across genotypes revealed by propensity normalization

We hypothesized that LPB and HPB may differ substantially at the transcriptome level. Temporal fluxes in the transcriptome serve as windows to both subtle and large-scale differences between the sibling introgression lines that could illuminate potential differences in global regulatory mechanisms. With propensity-normalized FPKM values (i.e., PS), we performed two comparisons to capture the profiles of transcriptome fluxes across genotypes and developmental stages. The first comparison utilized unfiltered PS, that is, total distribution ($-n < PS > +n$) within three windows of the flag leaf transcriptomes, namely, global or total gene set (n = 25,786), and transcription factor (n = 1,340) and stress-related (n = 2,589) gene subsets (Supplemental Figure S3; Supplemental Table S2). Hierarchical clustering indicated that in all three windows, the booting stage profiles exhibited significant dissimilarities between the four genotypes irrespective of growth condition. In contrast, LPB and HPB had very similar profiles at the vegetative stage under both irrigated and drought conditions with surprising similarity to WR, and dissimilarity to IR64 under irrigated condition. Fluxes at grain-filling had significant overlaps across genotypes, with LPB showing higher intensity in the positive propensity bands (Figure 2a). Similarities in vegetative profiles across LPB, HPB, and WR coupled with dissimilarity to IR64 was unexpected as the genomic contribution from WR was supposed to have been significantly diluted during recombination with Vandana and during the subsequent introgression to IR64 (Yadav et al., 2019).

The second comparison was based on filtered PS, which compared genes within the defined range of $-0.3 \leq PS \geq +0.3$, where the highest probabilities for significant differences in both positive and negative directions were expected (Supplemental Figure S3). Changes among these genes were not due to spurious fluctuations and included 8,215 genes, 410 genes, and 833 genes in the global, transcription factor, and stress-related windows, respectively. Hierarchical clustering more vividly demonstrated the uniqueness of fluxes in LPB at booting stage across the three windows (highlighted with red boxes), and recapitulated the similarities between LPB, HPB, and WR at the vegetative stage under irrigated conditions, dissimilarity with IR64 at vegetative stage, and overlaps between the four genotypes at grain-filling stage (Figure 2b). In summary, fluxes in LPB at booting stage had a well-modulated character under both irrigated and drought conditions for the vast majority of genes. In stark contrast, HPB, WR, and IR64 showed significant fragmentation of fluxes, giving evidence of a disjointed expression character (i.e., higher magnitude of perturbation). These patterns established the uniqueness of LPB at booting stage, especially relative to its sibling HPB.

3.3 | Unique patterns of transcriptome fluxes suggest superior productivity in LPB

Integral to adaptive responses at the cellular level, the directional character (i.e., upward skew, downward skew) of transcriptomic fluxes would be indicative of how well the complex waves of signals and gene activation and repression are

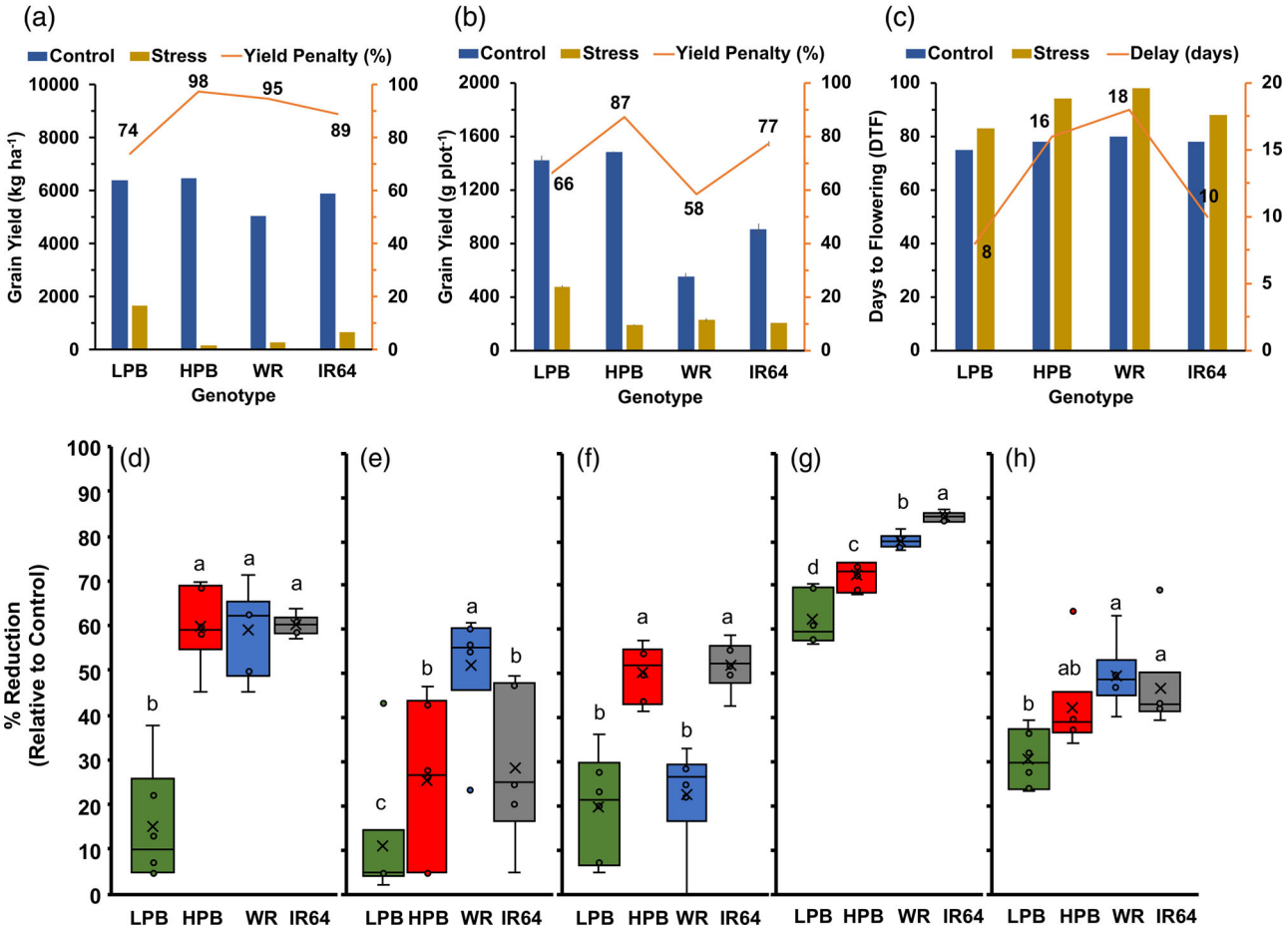

FIGURE 1 Synthesis and integration of all available data on relative agronomic and yield performances across the minimal comparative panel during progressive drought. Data from previous years of agronomic trials were integrated with the data collected from the 2017 wet season experiment performed for transcriptome studies. (a) Published grain yield results (GY; kg ha^{-1}) (Yadav et al., 2019) had significantly lower drought-mediated yield penalty (orange line) in LPB compared to the other genotypes. (b) Grain yield (g plot^{-1}, n = 3, means ± SE) from the 2017 wet season experiment recapitulated the trends in previous years (orange line). (c) Drought-induced flowering delay (orange line) from published results (Yadav et al., 2019) was also much less severe in LPB compared to the other genotypes. Trends in the (d) number of reproductive tillers per plant, (e) panicle length, (f) number of tillers per plant, (g) biomass per plant, and (h) plant height reiterated the superiority of LPB. Significant differences in flowering delay coupled with yield component reduction implied the earlier formation of reproductive sinks under drought in LPB, thus reducing grain yield penalty. LPB,low-yield penalty backcross introgression line; HPB, high-yield penalty backcross introgression line; WR, 'Way Rarem' (*qDTY12.1* donor); IR64, rice mega cultivar (recurrent parent). Box plots with similar letters are not statistically significant at $p < .05$ using Tukey HSD ($n = 6$)

organized in accordance with the underlying genetic circuitry towards cellular efficiency (Bechtold & Field, 2018). In conjunction with the flux analysis, we also determined the fraction of genes in the three flag leaf transcriptome windows with positive (PPF) and negative (NPF) Propensity, respectively. These genes were correlated with the magnitude of skewing of the Propensity distributions across developmental stages in all three windows (Figure 3; Supplemental Figure S3).

Consistent with the unique fluxes observed in LPB at booting stage, the directional characters of the three transcriptome windows were also unique in LPB at booting stage (red boxes in Figure 3a–c), with LPB exhibiting a downward skew (NPF > PPF) whereas HPB and WR had upward skews (PPF > NPF), and IR64 was neutral. With very few exceptions, the directional characters were highly conserved between the irrigated and drought conditions within a genotype, irrespective of developmental stage. This potentially "hard wired" nature of directional character signified that expression fluxes that correlate with either positive or negative phenotype may have resulted from fine-scale dynamics of transcriptional modulation within specific subsets of genes (i.e., networks). The downward directional character of LPB transcriptome at booting stage is an evidence of "tamed" responses, where fluxes are highly organized and targeted for effective use of the transcriptional machinery with much less trade-offs. In contrast, HPB and WR exhibited an "untamed"

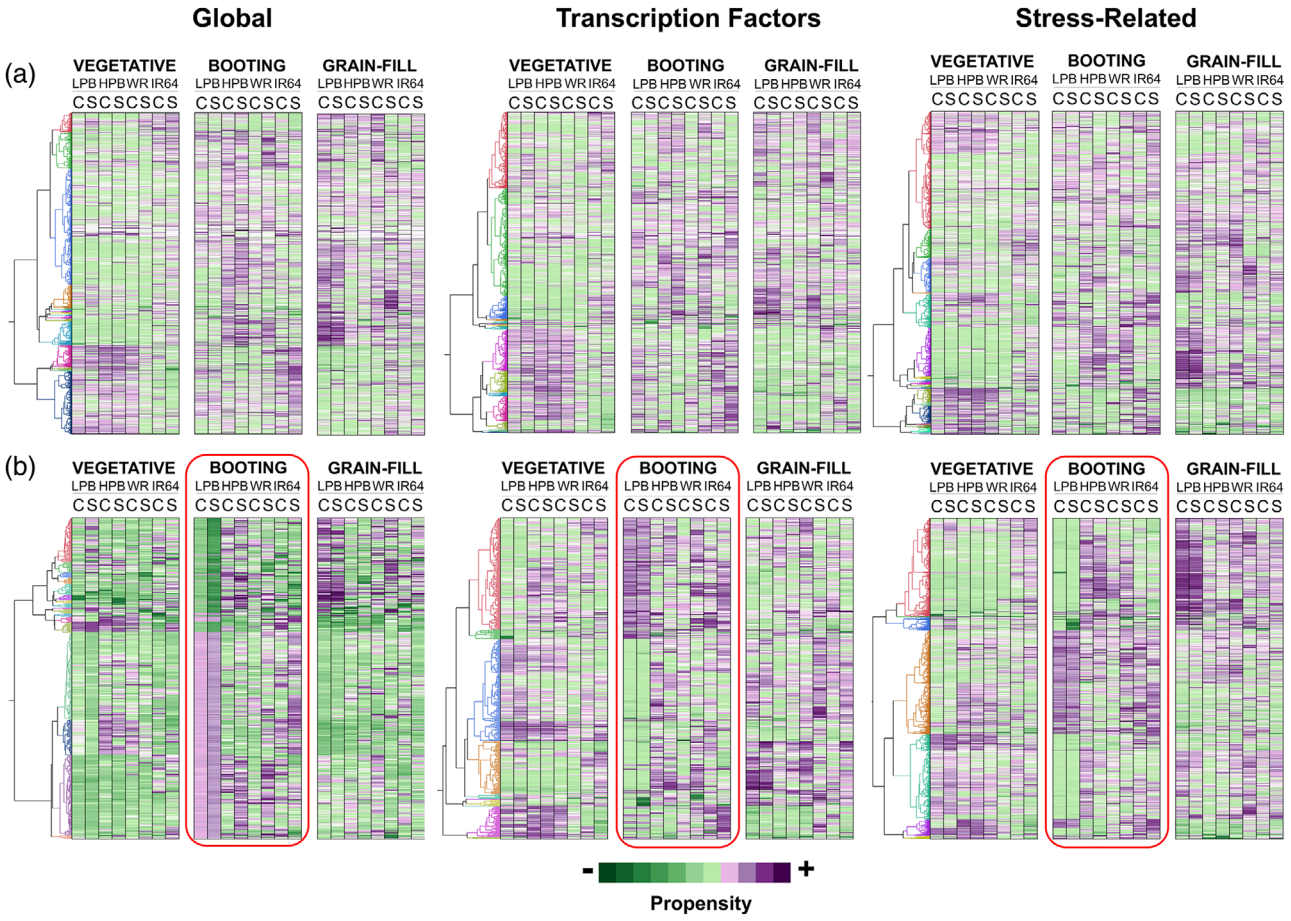

FIGURE 2 General trends in the transcriptomic fluxes across genotypes revealed by the filtered and un-filtered Propensity values of the global, transcription factor, and stress-related windows of the transcriptomes. (a) Hierarchical clustering of unfiltered global (27,786 loci), transcription factor (1,340 loci) and stress-related (2,589 loci) datasets. Expression fluxes at the vegetative stage under irrigated conditions highlight similarities between siblings (LPB, HPB) and 'Way Rarem' (WR) but not IR64. Expression fluxes at the booting stage revealed the uniqueness of LPB, while grain-filling stage fluxes revealed high similarities across all genotypes. (b) Filtered ($-0.3 \leq$ Propensity $\geq +0.3$) transcriptome datasets included 384 (global), 410 (transcription factor), and 833 (stress-related) gene loci. This comparison recapitulated the general trends in the unfiltered datasets and further underscored the uniqueness of LPB, particularly during booting (red boxes). On a locus-by-locus comparison, during booting stage in LPB appeared to be well conserved between irrigated and drought. Fluxes in HPB, WR, and IR64 reflected a state of perturbation. LPB, low-yield penalty backcross introgression line; HPB, high-yield penalty backcross introgression line

hence highly active and disordered response with potentially wasteful consequences (Bhogireddy et al., 2020; Fracasso et al., 2016).

Booting stage represents a critical shift in resource allocation from vegetative sources to reproductive sinks that could be impaired drastically by drought (Boonjung & Fukai, 1996; Zhang et al., 2018). Efficient use of cellular resources as mediated by the downward transcriptomic fluxes in LPB would prove beneficial for successful reproductive development. Unique signatures toward more modulated fluxes in LPB implies an efficient resource allocation that could be impacting source-sink strength toward reproductive transition (Supplemental Figure S2).

Coupled with the downward, more conservative fluxes at booting stage, LPB exhibited a positive skew across all three windows at grain-filling under drought (Figure 3a–c). This was in contrast to HPB, which had a negative skew in the same three windows (i.e., trade-off effects from compromised sink strength at reproductive transition). Both WR and IR64 had mostly nonskewed fluxes for all three windows under drought. Grain-filling stage represents the temporal continuum when the grain biomass is largely dependent on how well resources are channeled to reproductive sinks during development. Thus, upward transcriptomic fluxes in LPB compared with downward fluxes in HPB during grain-filling may have contributed to their differences in yield retention. The directional character of transcriptomic fluxes did not differ between genotypes at vegetative stage. However, there were differences in skew, with WR having the most downward fluxes (Figure 3a–c).

Evidence for the directionality trends was also apparent in the PS distribution plots of the global transcriptomes across

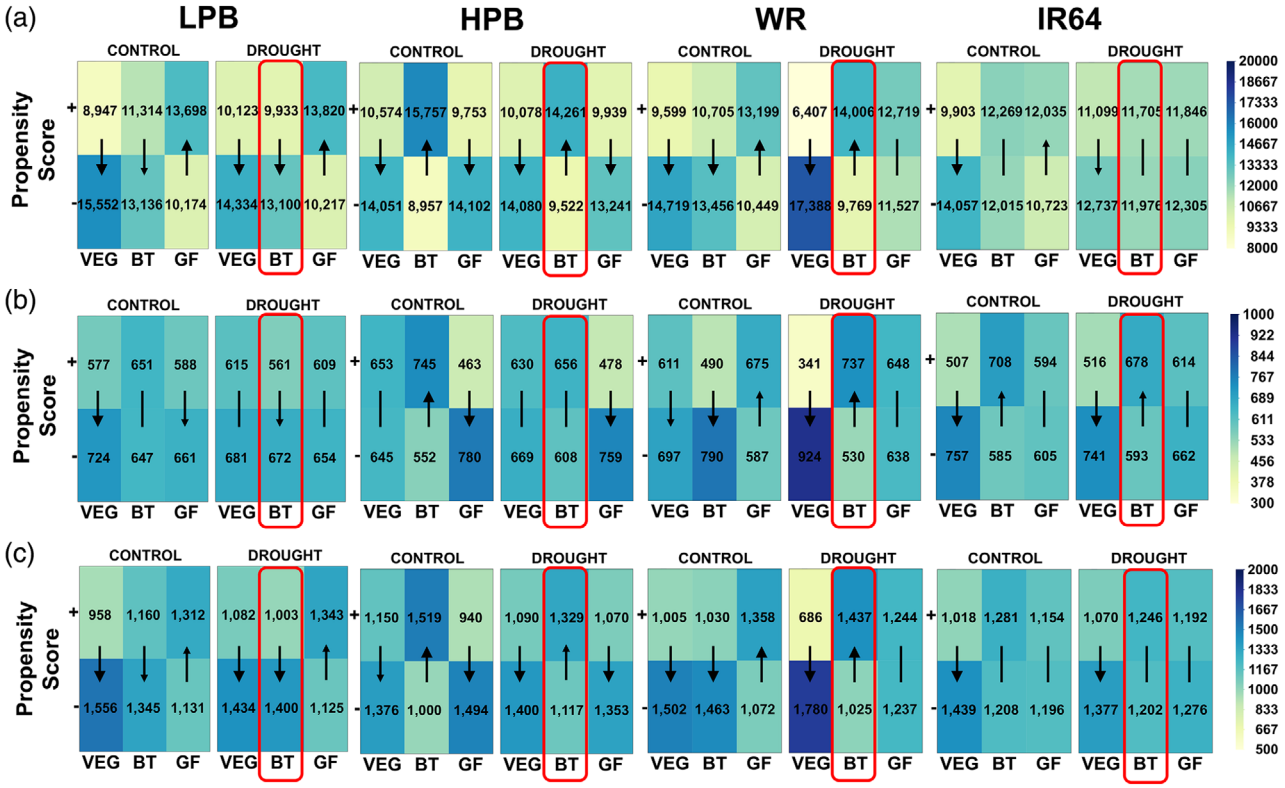

FIGURE 3 Differences in the directional character of transcriptomic fluxes across genotypes. Propensity scores of (a) global (25,786 loci), (b) transcription factor (1,340 loci), and (C) stress-related (2,589 loci) windows were divided into positive propensity (PPF) and negative propensity (NPF) fractions, excluding propensity = 0. Directional characters were positive skew (upward arrow; PPF > NPF), negative skew (downward arrow; NPF > PPF) or neutral (line without arrow). Global, transcription factor, and stress-related windows showed negative skew in LPB, positive skew in HPB and WR, and neutral in IR64 at booting stage under drought (red boxes). The downward directional character of the LPB transcriptome at booting under drought illustrated a tamed transcriptional landscape. Upward directional character in HPB and WR alluded to an untamed or noisy transcriptional landscape. LPB, low-yield penalty backcross introgression line; HPB, high-yield penalty backcross introgression line; WR, 'Way Rarem'; VEG, vegetative; BT, booting; GF, grain fill

genotypes (Supplemental Figure S3). The distribution plots at vegetative stage under irrigated condition had almost direct overlap across all genotypes, signifying that the transcriptomes were all in homeostatic, low-level conditions (i.e., no drastic perturbations). However, when drought was imposed and integrated with developmental signals, PS distribution plots began to diverge along the x-axis across genotypes. Skewing of PS plots matched the directional character of fluxes as determined by positive and negative PS fractions. Differences in expression fluxes and directional character of the flag leaf transcriptomes at booting stage indicated a unique drought response in LPB.

3.4 | Candidate yield-associated gene (*OsDEC*) encoded by *qDTY12.1*

It was proposed earlier that the major effect of *qDTY12.1* could be explained by a network of genes that regulate root architecture, coordinated by the transcription factor *OsNAM*$_{12.1}$ (Dixit et al., 2015). Although these findings represent a significant advance in understanding the function of *qDTY12.1* genes, evidence directly implicating this network to yield-related mechanisms is indirect at best. With the flag leaf transcriptome data, we re-examined the expression of all genes within the *qDTY12.1* defined by the syntenic region in the Nipponbare *RefSEquation* (chromosome-12) as delineated by the flanking RM28099 and RM511 markers (Dixit et al., 2012).

We found a total of 50 annotated protein-coding genes (Supplemental Table S4) within the syntenic 1.554 Mbp region in the Nipponbare *RefSeq* within coordinates 15,848,736 bp to 17,401,530 bp (Sakai et al., 2013). However, only 18 of these genes were expressed in at least one developmental stage in any genotype. Expressed genes occurred in small clusters interspersed with genes without detectable expression in the flag leaf (Figure 4). Co-expression analysis by RiceFREND (Sato et al., 2013) showed that none of the 18 expressed *qDTY12.1* genes formed networks amongst each other, suggesting that none of

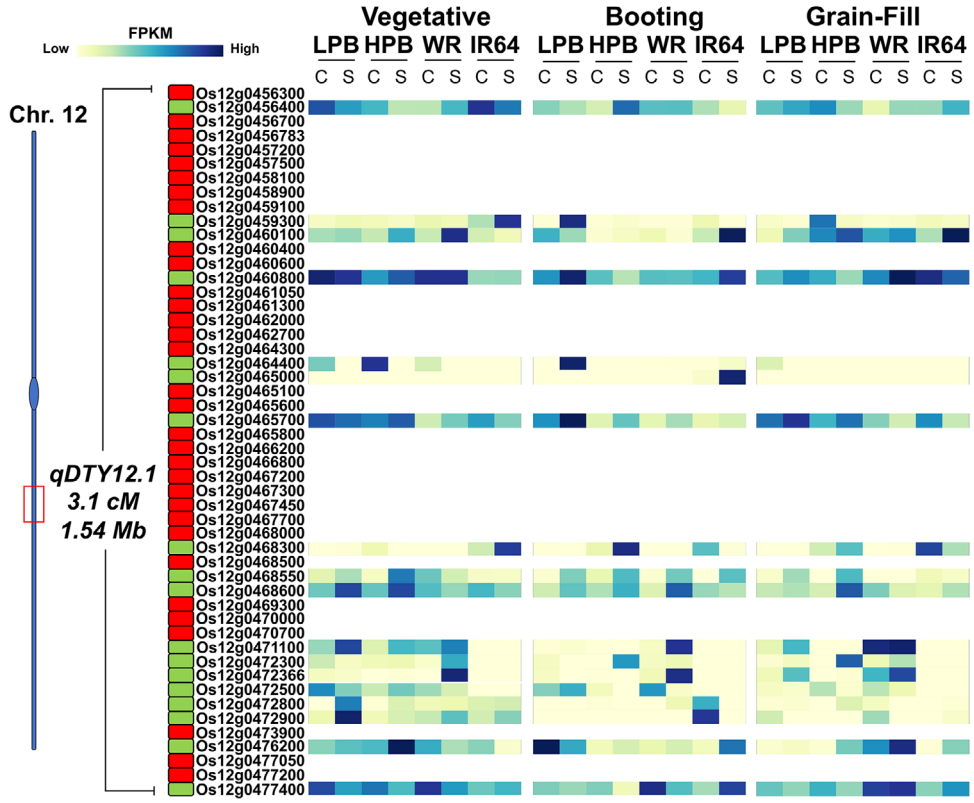

FIGURE 4 Expression profiles of *qDTY12.1* genes across the spatiotemporal windows of the transcriptomics experiments. Eighteen of the 50 *qDTY12.1* genes had measurable expression. The annotated protein-coding genes were organized by their location on chromosome 12 (*y*-axis) and their FPKM-based expression values (yellow = low; dark blue = high) and plotted across vegetative, booting, grain-filling stages under irrigated and drought conditions (*x*-axis). Expression is shown for genes with FPKM > 0 (green rectangles) and FPKM = 0 (red rectangles) under irrigated (C, control) or drought (S, stress) conditions. FPKM, fragments per kilobase of transcript per million; LPB, low-yield penalty backcross introgression line; HPB, high-yield penalty backcross introgression line; WR, 'Way Rarem'

them are in a common gene regulon as previously proposed (Dixit et al., 2015). However, the RiceFREND model showed that 12 of the genes had significant co-expression with other genes from across the genome outside the *qDTY12.1* boundary (Figure 5a).

One of the genes (*Os12g0465700*) was co-expresssed with two transcription factors (*Os05g0509400*, *Os08g0159800*) outside of *qDTY12.1* whose orthologs in Arabidopsis (*At3g22760* and *At1g32360*, respectively) are known to be involved in the regulation of cell division and expansion in the floral meristem (Andersen et al., 2007; Hauser et al., 2000; Klepikova et al., 2016; Sijacic et al., 2011; Wang et al., 2008). Designated as *OsDEC*, this gene was first reported as a regulator of leaf phyllotaxy and associated with shoot and root apical meristems through cytokinin signaling (Itoh et al., 2012). It was proposed that *OsDEC* functions as transcriptional regulator with broad spectrum targets in response to cytokinin-mediated growth signals (Bartrina et al., 2011; D'Aloia et al., 2011; Jameson & Song, 2016; Murai, 2014; Reguera et al., 2013; Wang et al., 2016; Zahir et al., 2001). The *OsDEC* gene was also implicated with reproductive and yield-related functions (Itoh et al., 2012).

The *OsDEC* gene was expressed in the flag leaf across developmental stages and genotypes, but differential induction by drought was evident from both the propensity-based and FPKM-based profiles. Drought-mediated upregulation of *OsDEC* was specific during the booting stage in LPB but not in HPB, WR, and IR64 (Figure 5b). The unique drought-induced expression of *OsDEC* in flag leaves of LPB at the critical stage of panicle development initiation further established its significance in the regulation of yield-related mechanisms (Inukai et al., 2005).

3.5 | Disruption of *DEC* orthologs in Arabidopsis compromised yield

Although there is a single copy of *OsDEC* (*Os12g0465700*) in the rice genome, duplicate copies (*At3G03460*, *At5G17510*) have been identified in Arabidopsis, with *At5G17510* as the closest ortholog (Supplemental Figure S4). Using the same design of the flowering-stage drought in the rice experiments (Supplemental Figure S2; Figure 6a), the T-DNA insertion mutants of *At3G03460* (3Gm) and *At5G17510* (5Gm) were

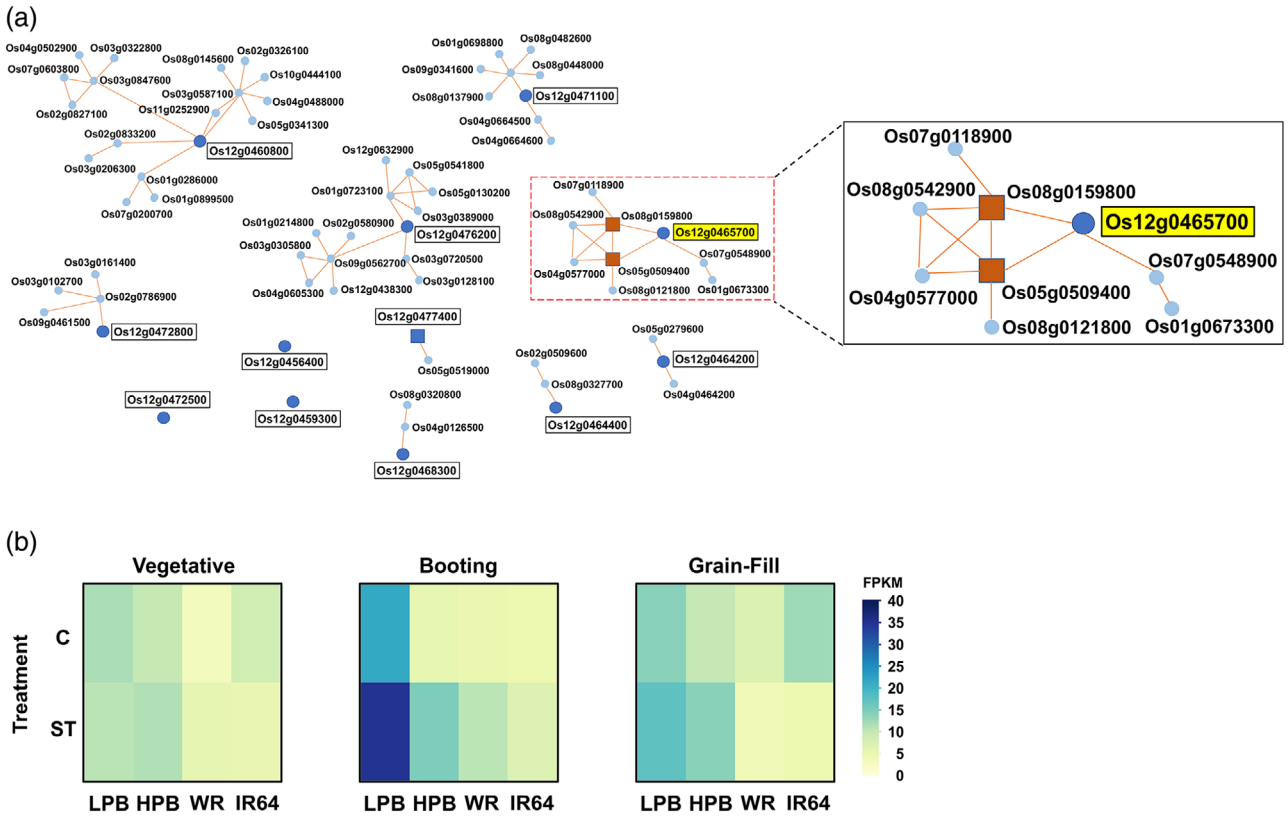

FIGURE 5 Co-expression of 18 *qDTY12.1* genes revealed by RiceFREND analysis. (a) None of the 18 expressed *qDTY12.1* genes had significant co-expression alliances with each other. However, 12 genes had co-expression alliances with genes outside of *qDTY12.1*, particularly in LPB. The *Os12g0465700* (*OsDEC*) had significant co-expression with two transcription factors (*Os08g0159800, Os05g0509400*) involved in floral meristem functions and singled out as the primary yield-related candidate gene. (b) *OsDEC* expression across the genotypic panel at vegetative, booting, and grain-filling stages under irrigated (control, C) and drought (stress, ST) conditions. *OsDEC* was induced by drought at booting stage only in LPB and first reported to have important roles in cytokinin signaling (Itoh et al., 2012). LPB, low-yield penalty backcross introgression line; HPB, high-yield penalty backcross introgression line; WR, 'Way Rarem'

compared with wild-type Col-0 in terms of agronomic performance. Expression was abolished in the T-DNA insertion mutants (Figure 6b). The 3Gm was very similar to Col-0 under irrigated conditions in terms of days-to-bolting, days-to-first-bloom, and days-to-seed-set (Supplemental Figure S5). On the other hand, 5Gm bolted much earlier and had shorter days to first bloom and seed-set relative to the wild-type. Strikingly, both 3Gm and 5Gm had significant yield penalties under drought at 34.5 and 41.9%, respectively, while Col-0 only had 13% yield penalty (Figure 6c, left panel). Dry biomass showed similar trends as in seed yield, with 3Gm having slightly higher biomass under irrigated condition compared with 5Gm and Col-0 (Figure 6c, right panel). However, there was no significant difference in the accumulation of biomass across the three genotypes under drought, suggesting that differences under drought were perhaps the result of altered source-sink dynamics in the mutants.

3.6 | *OsDEC* is the core of a network with other flowering-associated genes

Building on the RiceFREND analysis results of co-expression with two transcription factors with floral meristem function (Figure 5a), the flowering and yield test results under drought in *Arabidopsis* (Figure 6), we proceeded with using *OsDEC* as the foundation of a putative network related to flowering and therefore yield. The lack of apparent co-expression of *OsDEC* with other *qDTY12.1* genes suggested that if it was forming a network, the component genes would be located outside of the QTL boundaries. To address this hypothesis, we used *OsDEC* as bait to fish-out for other co-expressed genes in each genotype. In the first step of the iterative procedure, we used PS to identify the most significantly co-expressed genes at booting stage, revealing a total of 195 genes in LPB, of which the great majority were associated with cytokinin signaling.

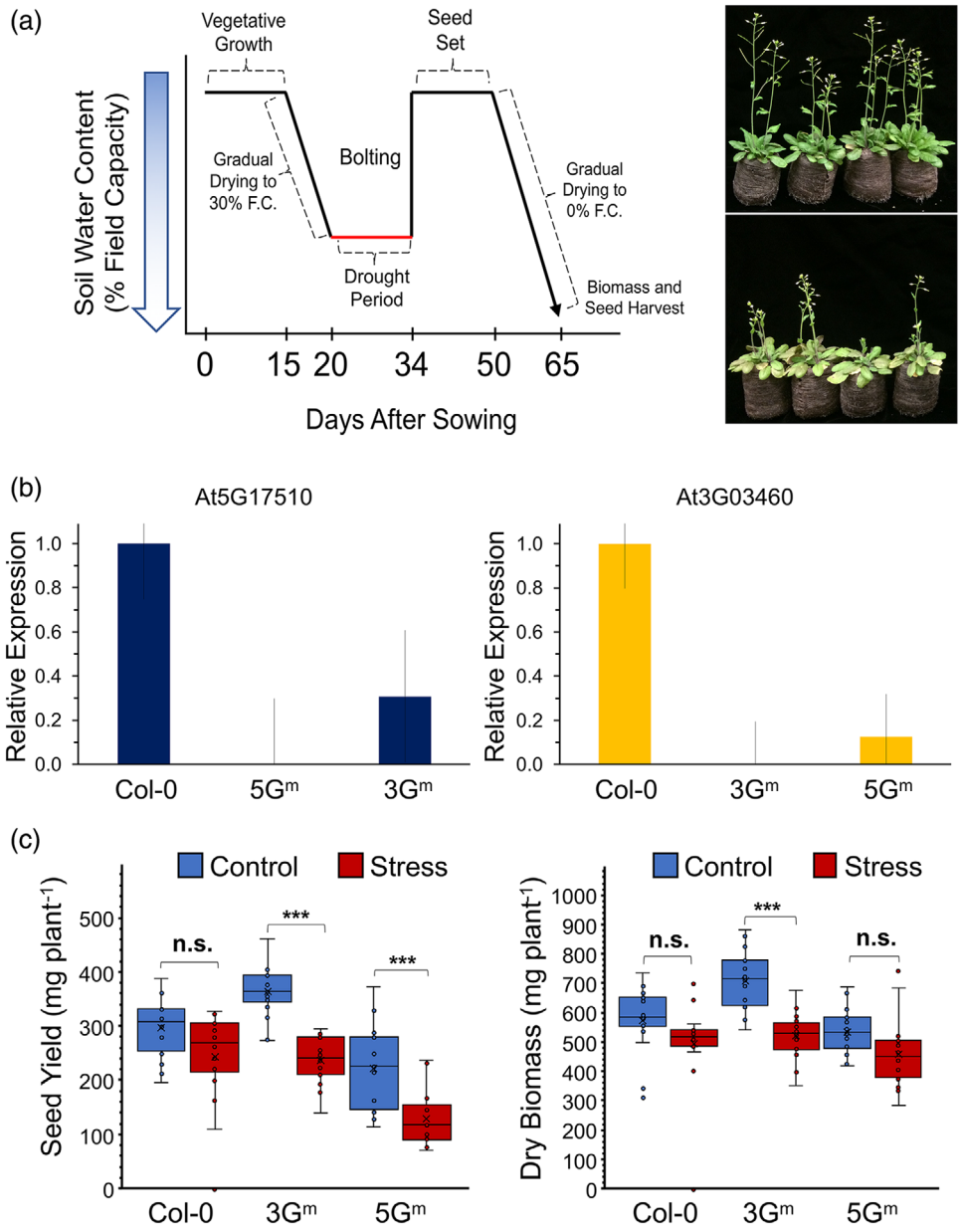

FIGURE 6 Direct significance of *OsDEC* to yield potential based on heterologous dissection of T-DNA insertion mutants of two orthologous gene copies. (a) Growth chamber drought experiments on *Arabidopsis thaliana* ecotype Col-0 and mutants (*At5G17510* = 5Gm, *At3G03460* = 3Gm) mirrored the designs of the drought experiments in rice (Supplemental Fig. S2). Drought was initiated 8 d before bolting (reproductive initiation) and lasted for 14 d, after which plants were rewatered to field capacity until maturity. (b) Transcript abundance analysis by qRT-PCR showing the silenced *At5G17510* (5Gm) and *At3G03460* (3Gm) relative to the expression in wild-type Col-0 at Day 14. (c) Boxplots showing the effects of the loss of *DEC* expression to plant biomass and seed yield. Significant reductions in seed yield under drought are evident in 5Gm and 3Gm ($p < .001$) but not in Col-0, while significant reductions in dry biomass under drought are evident in 3Gm ($p < .001$) but not in Col-0 and 5Gm. Post-hoc comparison of means (all pairs; $\alpha = 0.05$) was through significant ANOVA using Tukey-HSD: *** significant at $p < .001$. n.s., not significant

In the second step, the primary pool of co-expressed genes was further reduced to a much tighter cluster of 30 genes with the common gene ontology (GO) keywords of *cytokinin*, *flowering*, and *inflorescence* (Supplemental Figure 6A, red box). With a threshold PS of $n \geq 0.5$, the core of the network with the most significant similarities in flux with *OsDEC* was identified as a smaller subset of 11 genes (Table 1). The FPKM-based co-expression profiles of this core is shown in the hierarchical clustering in Supplemental Figure 6B, with Clades-2–4 exhibiting the most highly significant co-expression with *OsDEC* under both irrigated and drought conditions. *OsDEC* is a member of Clade-2 with three other genes (*Os07g0108900* = *OsMADS15*; *Os05g0521300* = *OsPHP3*; *Os03g0109300* = *OsLOGL3*). Annotation queries indicate

TABLE 1 OsDEC co-expression hub used as gene guides to extract booting stage network

Locus ID	Oryzabase gene symbol[a]	RAP-DB description[b]
Os02g0555300	*OsNAC28*	No apical meristem (NAM) protein domain containing protein
Os02g0830200	*OsRR3*	A-type response regulator, Cytokinin signaling
Os03g0109300	*LOGL3*	Similar to Lysine decarboxylase-like protein
Os03g0752800	*OsMADS14*	Similar to Isoform 2 of MADS-box transcription factor 14; APETALA1 (AP1)/FRUITFULL (FUL)-like MADS box transcription factor, Specification of inflorescence meristem identity
Os03g0810100	*OsIPT4*	Similar to tRNA isopentenyl transferase-like protein (Adenylate isopentenyltransferase)
Os05g0521300	*OsPHP3*	Similar to Histidine-containing phosphotransfer protein 4
Os07g0108900	*OsMADS15*	Similar to MADS-box transcription factor 15; APETALA1 (AP1)/FRUITFULL (FUL)-like MADS box transcription factor, Specification of inflorescence meristem identity, sexual reproduction
Os07g0568700	*OsFOR1*	Polygalacturonase-inhibiting protein, inhibitor of fungal polygalacturonase, regulation of floral organ number
Os08g0115800	*ONAC29*	NAC transcription factor, regulation of cellulose synthesis
Os10g0479500	*LOGL10*	Similar to carboxy-lyase
Os12g0465700	*DEC*	Plant-specific protein containing a glutamine-rich region and a conserved motif, controls of phyllotaxy by affecting cytokinin signaling

[a]Oryzabase (Integrated Rice Science Database; https://shigen.nig.ac.jp/rice/oryzabase/).
[b]The Rice Annotation Project Database (RAP-DB; https://rapdb.dna.affrc.go.jp/index.html).

that these genes shared common functions by virtue of their roles in the specification of *inflorescence meristem identity* (GO:0048510), *floral organ regulation* (GO:0048833), *cytokinin signaling* (GO:0009736), and *cytokinin biosynthesis* (GO:0009691). The only gene in Clade-3 was annotated as a floral organ regulator (*Os07g0568700*; GO:0048833). The other solitary gene in Clade-4 is annotated as *OsMADS-14* (*Os03g0752800*), which functions in the specification of inflorescence meristem identity (GO:0048510).

To capture the secondary and tertiary components of the *DEC*-network, other genes with cytokinin-associated functions that exhibited significant co-expression with *OsDEC* and/or its five other direct cohort genes were identified in the third iteration. The FPKM-based hierarchical clustering revealed a larger group that formed 13 clades of tightly co-expressed genes around the *DEC*-network. A total of 36 genes (Supplemental Table S5) that were most significantly co-expressed with *OsDEC*, and its direct cohort genes were contained within two clades that reflect the potential functional significance of the *DEC*-network (Figure 7a). The main hub of this network of 36 genes is *OsDEC* itself and two MADS-box transcription factors that regulate meristem transition from vegetative to flowering stage (i.e., *Os07g0108900* = *OsMADS15*, *Os01g0922800* = *OsMADS51*) (Figure 7b). The other peripheral components surrounding the *DEC*-network were dispersed across seven clades, all of which are associated with vegetative to reproductive transition of the meristem.

3.7 | *DEC*-network is specific to booting stage and genotype-dependent

To further understand the potential significance of *OsDEC* to yield maintenance under drought, we compared the *DEC*-network organization across developmental stages within LPB (i.e., vegetative vs. booting vs. grain-filling) and across genotypes with or without the *qDTY12.1* (i.e., LPB vs. HPB, donor parent WR, and recipient parent IR64). Hierarchical clustering showed significant differences in co-expression among the 36 core and peripheral genes of the *DEC*-network across developmental stages (Figure 7c–e). In LPB, genes of the *DEC*-network were coordinately induced by drought specifically at booting stage, while no significant changes in expression were detected at vegetative and grain-filling stages.

Further examination of the organization of the booting-stage network across genotypes revealed widely divergent patterns, with only LPB showing evidence of coordinated expression of all core and peripheral components (Figure 8a). The genotype-dependent and booting stage-specific signatures in LPB suggested that the operability of the *DEC*-network was likely a consequence of the proper alignment of all the upstream regulatory components that established the optimal expression of *OsDEC* and, subsequently, all of its downstream cohort/peripheral genes.

The disorganized *DEC*-network in HPB appeared to suggest the opposite of what was observed in LPB, perhaps due to the lack of complementary alleles for the upstream

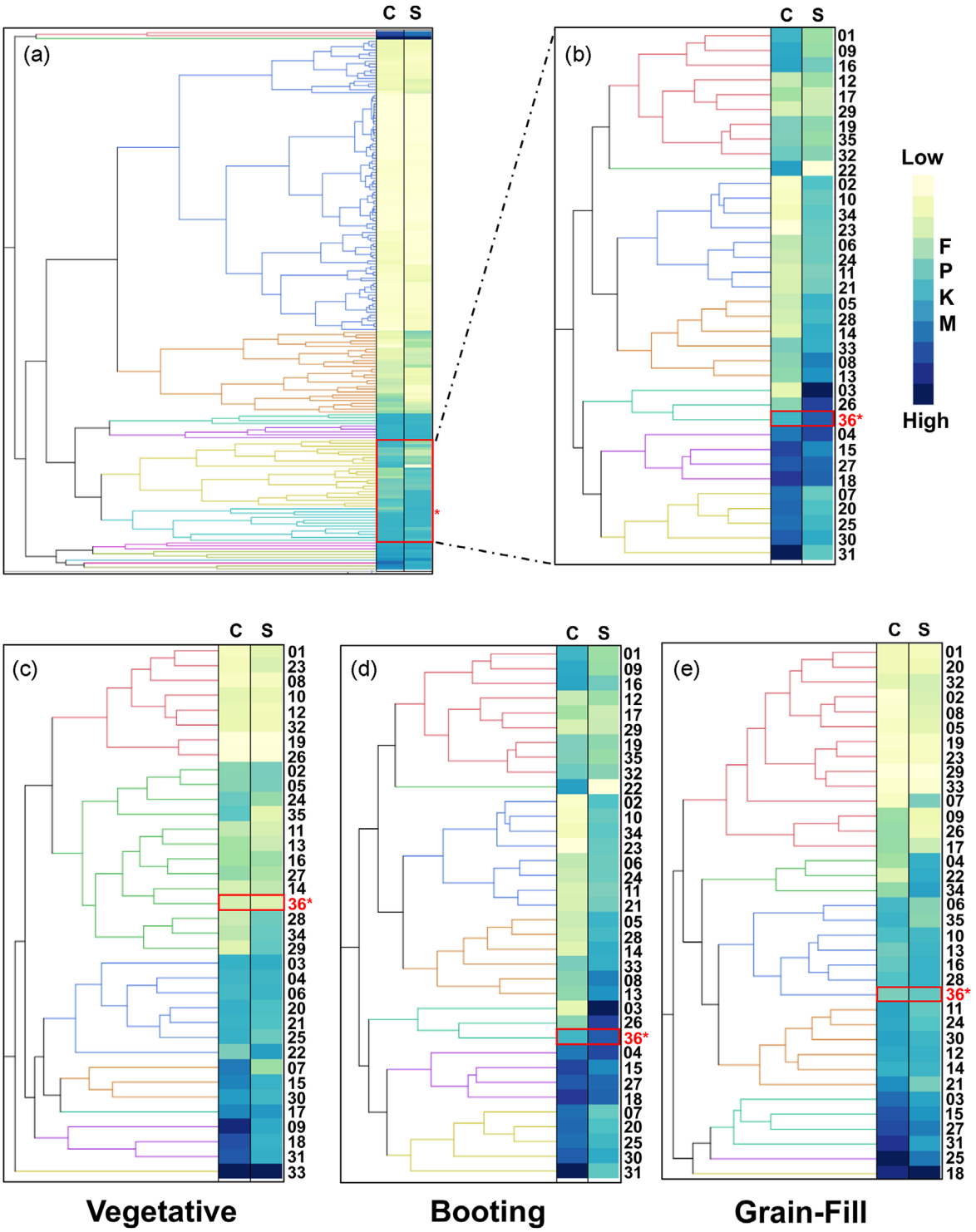

FIGURE 7 Differential organization of *DEC*-network showing the uniqueness of LPB. High similarities in *OsDEC* network were evident across all genotypes at vegetative and grain-filling stages. (a) Hierarchical clustering of FPKM-based transcript abundances revealed 13 clades of co-expressed genes surrounding the *DEC*-network hub. Clades-7 and −8 (red box with asterisk) contained 36 genes that were highly co-expressed with *OsDEC*. (b) Final composition of the *DEC*-network of LPB based on hierarchical clustering of FPKM-based transcript abundances. The 'core' of the network consisted of *OsDEC* (36*), *OsMADS15* (26), and*OsMADS51* (03), all of which are directly involved with meristem transition from vegetative to reproductive. The other 33 genes formed the peripheral components with direct linkages to reproductive functions. (c-e) Hierarchical clustering of FPKM-based transcript abundances across the 36-member *DEC*-network. Numbers to the right of dendrograms (Locus ID, position, annotation, etc.) are detailed in S4 Table. Red asterisk marks the position of *OsDEC*. C, control/irrigated; S, stress/drought; FPKM, fragments per kilobase of transcript per million

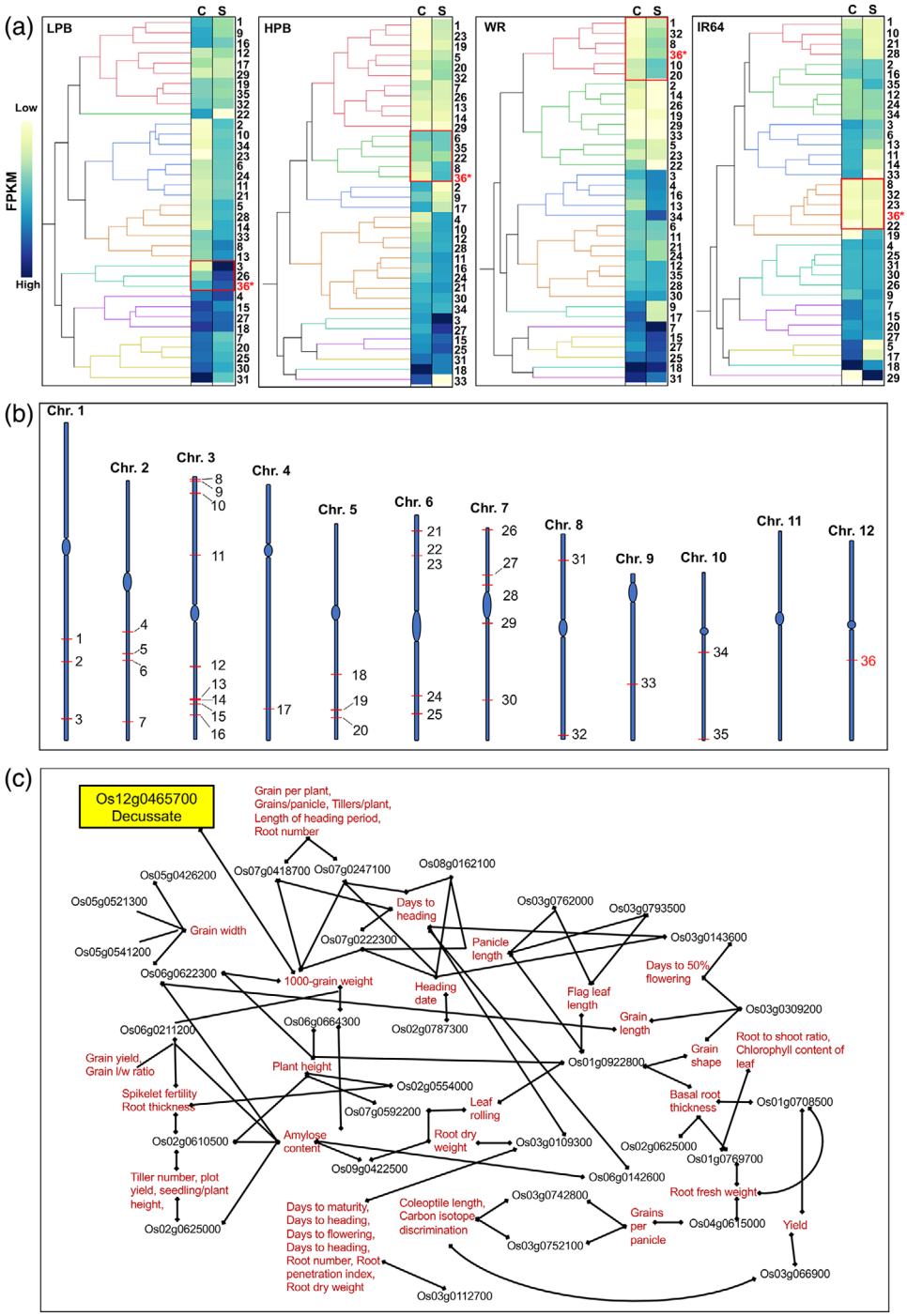

FIGURE 8 Organization of the booting stage *DEC*-network across genotypes. The *OsDEC* formed networks with other genes in the genetic background, and the network is highly organized in LPB but not in the other genotypes, where homologous networks appeared fragmented and disorganized. (a) The organization and expression character of the *DEC*-network at booting stage are distinct in each genotype. In LPB, the network is characterized by an inductive pattern while a static pattern was evident in HPB, WR, and IR64. (b) Distribution of the members of the functional *DEC*-network across the rice genome outside of *qDTY12.1*. Numbers to the right of the dendrograms are described (Locus ID, position, annotation, etc.) in Supplemental Table S4. Number with red asterisk indicate the position of *OsDEC*. C, control/irrigated; S, stress/drought; LPB, low-yield penalty backcross introgression line; HPB, high-yield penalty backcross introgression line; WR, 'Way Rarem'

components that facilitate the same level of network organization as observed in LPB. The variant patterns in the *DEC*-network between LPB and HPB, both of which had the same *OsDEC* allele from WR (sequence validation data not shown), further implied an efficient integration of stress and developmental signals through the interaction between *qDTY12.1* and its peripheral cohort genes in the genetic background (Figure 8b). Thus, a complete *DEC*-network appeared to be strategic to an optimal integration of drought-response and developmental signals during the early stages of flowering when the critical reproductive sink is being established.

3.8 | Yield component traits associate with the *DEC*-network

For further interpretation of the larger biological significance of the *qDTY12.1*-encoded *OsDEC* and its network with other genes in the genetic background, we established a biological network map through the Knetminer knowledge integration tools (Hassani-Pak, 2017). This analysis links many pieces of relevant information from all types of genetic studies curated in the literature to establish direct or indirect associations between a gene or network of genes and physiological and agronomic traits. The knowledge integration map directly linked all but one of the 36 genes that comprised the *DEC*-network with various yield component traits, particularly those relevant to source-sink regulation, sucrose and starch biosynthesis and deposition, grain-filling, seed development and maturation, and seed weight (Figure 8c; Supplemental Table S6).

A recent study in maize (*Zea mays* L.) highlighted the significant impacts of *ZMM28* overexpression to flowering time, plant growth, photosynthetic capacity, nitrogen utilization, and yield under drought (J. Wu et al., 2019). *ZMM28* is a member of the AP1-FUL subgroup of MADS-box transcription factors with critical roles in the regulation of flowering time, floral organ identity, and vegetative to reproductive transition (Becker & Theißen, 2003; Kater et al., 2006; Ng & Yanofsky, 2001). We found that *OsMADS18* (*Os07g0605200*), the closest ortholog of *ZMM28* in rice, along with two other MADS-box genes (*Os03g0752800* = *OsMADS14; Os07g0108900* = *OsMADS15*) had strikingly similar expression as *OsDEC* in LPB at the booting stage (Figure 9). Expression peaked at booting stage in LPB and IR64, but not in HPB and WR. These findings suggested the influence of IR64 genetic background in the optimal configuration of *DEC*-network in LPB but not in HPB. Of important note, the expression of *OsMADS18* in LPB across developmental stages was very similar to the *zmm28* signature in transgenic maize (Wu et al., 2019).

Interestingly, LPB had the shortest delay (8 d) in flowering time under drought in comparison to IR64, HPB, and WR, with 10-, 16-, and 18-d delay, respectively (Figure 1). Coupled with the observed trends in MADS-box expression, it appeared that the *DEC*-network in LPB had integrated the function of *OsMADS14*, *OsMADS15*, and *OsMADS18* toward a mechanism for reducing the time delay in reproductive growth transition during drought.

4 | DISCUSSION

Introgression of large-effect QTLs such as *qDTY12.1* have shown major incremental improvements in rice yield maintenance under drought (Bernier et al., 2007; Ghimire et al., 2012; Kumar et al., 2014; Mishra et al., 2013; Sandhu et al., 2014; Singh et al., 2016; Vikram et al., 2011, 2016). However, there have been instances when introgression did not confer the expected phenotypic effects (Yadav et al., 2019). A similar phenomenon has been reported on *SalTol* for salinity tolerance in different rice cultivars, when introgression of the QTL alone did not necessarily lead to the expected phenotypic effects (Han et al., 2020; Pabuayon et al., 2021). Inconsistencies are caused by negative or positive epistatic interactions between the QTL genes and other genes in the genetic background that could either enhance or drag the QTL effects (de los Reyes, 2019; Pabuayon et al., 2020). We further illuminated this enigma by integrating the concept of the Omnigenic theory (Boyle et al., 2017) and by using transgressive mechanisms to support our conceptual framework (de los Reyes, 2019; Pabuayon et al., 2021; Pabuayon et al., 2020).

4.1 | Significance of *qDTY12.1* to genetic network rewiring

It was postulated that nonparental traits created by genetic recombination are due to genetic coupling–uncoupling and network rewiring effects. Rewired genetic networks are caused by large assemblages of synergistic or antagonistic alleles that get coupled or uncoupled during multiple rounds of recombination (de los Reyes, 2019; Pabuayon et al., 2021; Pabuayon et al., 2020). In the context of the Omnigenic theory, the few core genes/alleles with major effects on phenotypic variance could either be coupled or uncoupled with numerous compatible or incompatible peripheral genes/alleles with minute but additive effects on the phenotypic variance (Boyle et al., 2017). The additive effects of peripheral genes/alleles across the genetic background may either enhance or drag the effects of the core genes that function as the hub of the network.

Our results showed yet another layer of evidence that the inconsistent effects of *qDTY12.1* observed across two sibling introgression lines in the genetic background of IR64 were due to either optimally (LPB) or suboptimally (HPB) rewired

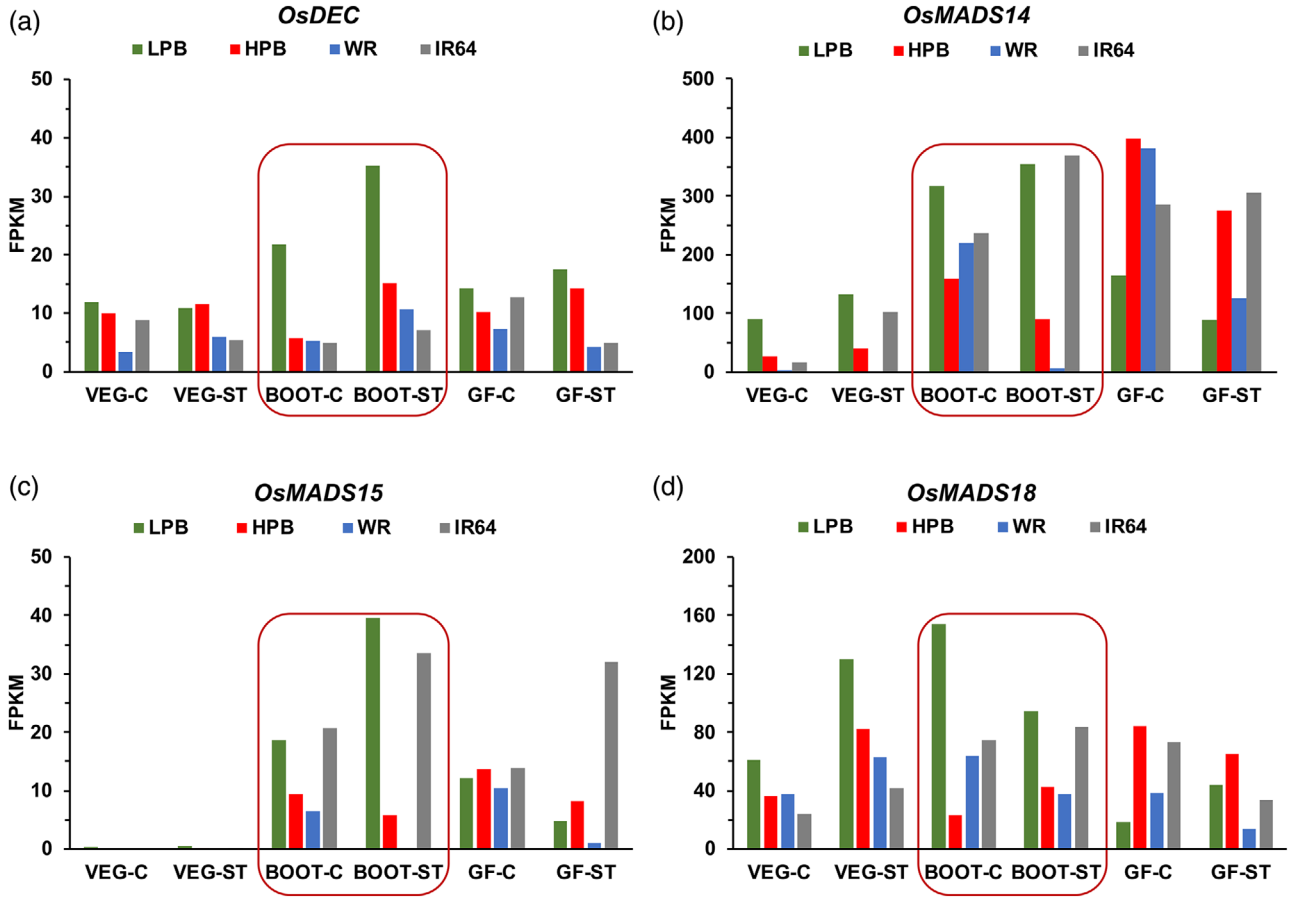

FIGURE 9 Expression of critical MADS-box transcription factors at booting stage in LPB mimic the signature of *OsDEC*. The temporal expression of *OsDEC* and three MADS-box transcription factors point to a mechanism for regulating flowering-time under drought. (a–d) FPKM-based expression plots of *OsDEC*, *OsMADS14*, *OsMADS15*, and *OsMADS18* across growth conditions (irrigated, drought) and developmental stages. *OsMADS14*, *OsMADS15*, *OsMADS18* (documented to be intimately involved in flowering and meristem identity) were induced by drought at booting stage (red boxes) in LPB and IR64, but not in HPB and WR. Expression of *OsMADS18* across developmental stages mimicked the overexpression (OE) of *ZMM28* (maize ortholog) that led to improved growth and yield (Wu et al., 2019). VEG-C, vegetative control/irrigated; VEG-ST, vegetative stress/drought; BOOT-C, booting control/irrigated; BOOT-ST, booting stress/drought; GF-C, grain-filling control/irrigated; GF-ST, grain-filling stress/drought; FPKM, fragments per kilobase of transcript per million; LPB, low-yield penalty backcross introgression line; HPB, high-yield penalty backcross introgression line; WR, 'Way Rarem'

genetic networks, with a *qDTY12.1*-encoded regulatory gene *OsDEC* functioning as the hub of the network. We hypothesize that while backcross introgression of the functional *qDTY12.1* allele from WR into IR64 genetic background (through a bridge donor derived from WR × Vandana) may have preserved the integrity of the original *qDTY12.1* allele by marker-assisted selection of the foreground, the genomic environments (background) of the introgressed *qDTY12.1* were likely to be significantly divergent between sibling introgression lines. By extension, the rewired genetic networks were configured by many other genes/alleles from either parents, organized in such a manner that either optimal or suboptimal alliances define the operative structure of the network. Further, the superior progeny (LPB) appeared to contain not only the required network hub (i.e., *OsDEC* allele from WR) but also the optimal assemblage of peripheral alleles across the genetic background leading to a fully functional synergy. These peripheral alleles are likely to have come directly either from IR64 or remnant and cryptic introgression of alleles from WR or Vandana that escaped the resolution and scope of marker-assisted selection of the background genome. On the other hand, while the inferior sibling HPB also contained the identical network core from WR, it appeared to be lacking the same optimal assemblage of peripheral alleles from the genetic background to configure a functioning *DEC*-network (Supplemental Figure S1).

Comparative dissection of the flag leaf transcriptomes of LPB and HPB in relation to the *qDTY12.1* donor parent WR and recipient parent IR64 showed that while the global patterns under irrigated condition at the vegetative and grain-filling stages were generally similar across the genotypes, there were drastic differences at the booting stage (Figure 2).

These differences appeared to be the result of coupling–uncoupling effects, hence interaction of distinct subsets of synergistic and antagonistic alleles from either parent. As such, the positive effect of *qDTY12.1* introgression in context of the *DEC*-network would be manifested only when an optimal number of compatible peripheral alleles with additive effects are assembled to generate the transgressive genetic network that was apparent in the booting-stage transcriptome of LPB. It is evident, based on distinct transcriptomic signatures of LPB and HPB, that while *qDTY12.1* has a large effect on yield, expressing its full potential requires many other peripheral genes across the genetic background.

Our current results do not indicate that any other genes within the *qDTY12.1* are important for the full functionality of the *DEC*-network. Indeed, all of the 35 peripheral genes that comprised the functional *DEC*-network in LPB are dispersed throughout the genome, clearly outside of the boundaries of *qDTY12.1* (Figure 8b). Thus, the transgressive nature of yield maintenance under drought, as conferred by *OsDEC*, requires a synergy with many other genes in the genetic background. This was made abundantly clear by the fact that although HPB and WR had the same *qDTY12.1* allele as LPB, their yield potentials under drought were woefully inferior.

Another important advance contributed by this study is the discovery that while LPB and HPB were assumed to be largely similar with regard to *qDTY12.1*, the global flag leaf transcriptome of LPB specifically at booting stage was drastically different from its recurrent and QTL donor parents and sibling introgression line (Figure 2b). Booting stage represents a critical crossroad of photosynthetic source-sink dynamics between the flag leaf and developing inflorescence, characterized by physiological and biochemical processes that sustain seed development (Abdalla Basyouni Abou-Khalifa et al., 2008; Counce et al., 2000; Cui et al., 2003; Rahman et al., 2014; Yoshida, 1981) (Supplemental Figure S2). As such, events unique to LPB at booting stage provides a valuable link to the functional significance of *qDTY12.1* to cellular mechanisms critical to yield components. It has been shown that the timing of drought is most deleterious at the initiation of booting, with negative effects on yield-related traits including grain number per panicle, panicles per area, and total above ground biomass (Zhang et al., 2018). The significance of *qDTY12.1* is consistent with the synchronized activation of the *DEC*-network when drought coincides with the early stages of floral organ development (Bernier et al., 2007, 2009; Henry et al., 2015; Torres & Henry, 2018).

4.2 | *OsDEC* affects yield-related processes likely through cytokinin signaling

Based on its unique drought-induced expression in the flag leaf of LPB only, specifically at the initiation of booting, *OsDEC* was identified as the most likely candidate from within *qDTY12.1* for yield-related functions. Similarly, we found that among the 18 flag-leaf transcribed *qDTY12.1* genes, only *OsDEC* was differentially induced by drought at the booting stage and exhibited significant co-expression with key transcription factors involved in reproductive transition (Figure 4, Figure 5a–b). While the specific biochemical function of *OsDEC* remains unknown, it is known to have a regulatory function over Type-A and Type-B response regulators in the two-component cytokinin signal transduction pathway (Hill et al., 2013; Itoh et al., 2012; To et al., 2004; Xie et al., 2018). Cytokinin is intrinsic to a myriad of cellular processes that are critical for seed development as well as for mediating cellular signals in response to drought (Ashikari, 2005; Bartrina et al., 2011; Jameson & Song, 2016; Murai, 2014; Peleg et al., 2011; Reguera et al., 2013; Zahir et al., 2001). Studies in many agronomically important crops have also shown that overexpression of cytokinin biosynthetic genes leads to significant improvements in yield potential under drought (Kuppu et al., 2013; Qin et al., 2011; Wang et al., 2016; Zhu et al., 2018).

The results of this study support a hypothesis that through a cytokinin-mediated pathway, *OsDEC* regulates physiological processes in the flag leaf that appear to be important in adjusting the timing of floral organ initiation when the photosynthetic source is perturbed or progressively exhausted by drought. We postulate further that this mechanism could be important in ensuring the early establishment of a strong reproductive sink to sustain the requirements of seed development and maturation when resources continue to be limited by drought effects. Indeed, the 35 member genes of the *DEC*-network were mostly regulatory with key functions in floral meristem, vegetative to reproductive transition, cytokinin signal transduction, and other aspects of reproductive growth. These trends were further reiterated by the models generated by KnetMiner, which showed that all genes in the larger *DEC*-network funnel into processes involved with seed development, grain filling, sucrose transport, starch biosynthesis, and many other yield-component traits (Figure 8c).

Furthermore, many introgression lines of *qDTY12.1* have been extensively studied to determine what physiological characteristics are important in the maintenance of low-yield-penalty under drought (Henry et al., 2014, 2015; Raorane, Pabuayon, Miro et al., 2015). These characteristics include water uptake efficiency, increased proline levels in roots, improved remobilization of amino acids for nitrogen status, improved transpiration efficiency, increased panicle branching, increased lateral root formation, and a reduction in flowering delay under drought. These trends are consistent with the proposed significance of *OsDEC* in integrating survival, developmental, and stress-related responses to minimize the

cost of cellular perturbations to reproductive growth (Yadav et al., 2019).

From the standpoint of productivity, flowering represents a developmental crossroad. As such, it is regulated tightly by environmental signals to ensure reproductive success of the species, hence the process is dynamic, multifaceted, and with multiple levels of control over a large number of genes. A closer examination of the components of functional *DEC*-network (i.e., 35 genes) indicate direct connections to one or more molecular, cellular, or biological functions that are relevant to the control of flowering time, including hormonal signaling (GO:0007267), light signaling (GO:0009416), epigenetic control (GO:0040029), developmental control of floral organ differentiation and fate (GO:0048437), maintenance of reproductive meristems (GO:0010073), and transcriptional regulation (GO:0006357). Some of the well-known MADS-box transcription factors, such as *OsMADS14*, *OsMADS15*, and *OsMADS18*, define the hallmark signatures of direct association of *OsDEC* with the regulation of flowering time (Fornara et al., 2004; Kater et al., 2006; Lee & An, 2015).

The magnitude of drought-induced delay in flowering is strongly correlated with yield retention in rice (Kumar et al., 2009; Pantuwan et al., 2002). Progressive drought imposed before the onset of flowering caused 8-, 16-, 18-, and 10-d delays in normal flowering time in LPB, HPB, WR, and IR64, respectively (Figure 1). Under limited water conditions, earlier flowering would provide a developmental adjustment to minimize the effects of continuous depletion of photosynthetic sources that would normally sustain robust reproductive transition. Therefore, expression of many flowering-related genes with molecular and cellular functions associated with floral organ identity (GO:0010093), inflorescence meristem maintenance (GO:0010077), and spikelet development (GO:0009909) appeared to commence earlier in LPB due to drought. These GO terms are relevant to the establishment and maintenance of critical yield-component traits such as number of fertile spikelets, number of reproductive tillers, number of panicles, grain weight, number of grains per panicle, and panicle size, as verified by yield components data (Figure 1, Figure 8c).

4.3 | Potential implications of *DEC*-network at the molecular and cellular levels

In earlier efforts to characterize the cellular functions of *OsDEC* using *dec* mutants, the following conclusions emerged: (a) *OsDEC* is insensitive to exogenous cytokinin; (b) *OsCKX2* and other *cytokinin oxidase* genes were upregulated in knock-out mutants; (c) active cytokinins cZ and iP, along with some of their intermediates were significantly reduced in mutants; (d) expression of *Lonely GUY* genes were not affected in mutants; and (e) Type-A response regulators were downregulated while some Type-B response regulators were upregulated (Itoh et al., 2012). The DEC protein potentially functions as a transcriptional regulator based on the N-terminus glutamine-rich domain associated with chromatin remodeling functions (Ding et al., 2006; Freiman & Tjian, 2002; Rahman et al., 2011; Saluja et al., 1998; Wu & Chiang, 2007). By integrating these pieces of information with other co-expressed genes in LPB, we propose a hypothetical model of the mechanisms by which the *DEC*-network could regulate early flowering (Figure 10).

We hypothesize that in LPB, the pools of active cytokinins would be enhanced as indicated by the drought-mediated upregulation of *OsLOGL3* and *OsLOGL7* (*Lonely Guy*) and downregulation of *OsCKX2*. The significance of *OsCKX2* downregulation to the enhancement of grain yield in rice has been confirmed (Ashikari, 2005). In the model, the pool of active cytokinin is upregulated with concomitant downregulation of cytokinin degradation by *OsCKX2*. Studies have shown that *OsDEC* regulates *CKX* expression but not *Lonely GUY* expression (Itoh et al., 2012). It has also been reported that a drought- and salinity-associated C2H2 zinc-finger transcription factor (*OsDST*) is directly involved in the regulation of *OsCKX2* (Huang et al., 2009). The *DST* mutation ($OsDST^{reg1}$) downregulates *OsCKX2*, thereby increasing the level of active cytokinin (Li et al., 2013). Downregulation of *OsDST* was evident in the flag leaf transcriptome at the booting stage, with −2.2 and −5.9 log2-fold decreases in transcript abundance under drought in LPB and IR64, respectively. In contrast, *OsDST* was upregulated in HPB and WR with 0.74 and 0.62 log2- fold changes, respectively (Supplemental Figure S7).

Increased levels of active cytokinin have been implicated to yield enhancement in rice, which correlates well with the higher yield potential of LPB under drought and parallel upregulation of cytokinin biosynthetic genes and downregulation of cytokinin degradation genes, such as *OsCKX2* (Bartrina et al., 2011; Jameson & Song, 2016; Murai, 2014). In addition, cytokinin signaling directly affects other genes that regulate flowering (D'Aloia et al., 2011; El-Showk et al., 2013; Hwang et al., 2012; Zürcher & Müller, 2016). Accumulation of active cytokinin in LPB suggests a mechanism that facilitates earlier induction of flowering under drought as a penalty-avoidance response by establishing proper source-sink dynamics earlier before the source becomes more limited or depleted.

Networks of *OsDEC* with *OsMADS14*, *OsMADS15*, and *OsMADS51* showed that indeed the flowering pathway was induced earlier in LPB. These MADS-box transcription factors are critical for regulating inflorescence meristematic processes in rice (Kater et al., 2006; Kim et al., 2007; Lee & An, 2015; Weng et al., 2014). A recent study in maize also showed that overexpression of the *OsMADS18* ortholog in maize (*zmm28*) led to significant increases in yield under

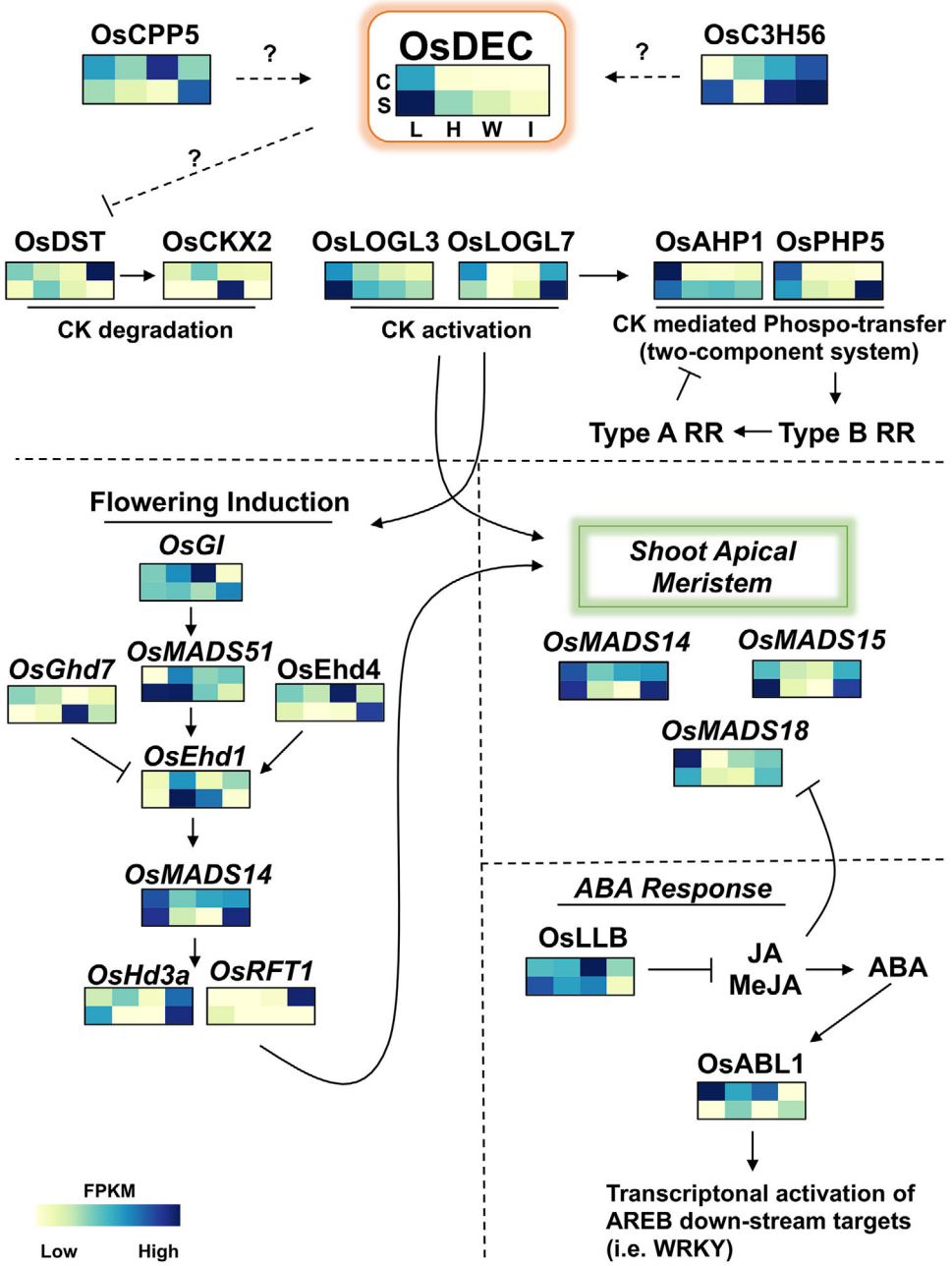

FIGURE 10 Putative molecular mechanism of the *DEC*-network modeled through the integration of relevant information from the literature with the trends uncovered from the flag leaf drought transcriptomes. In concert with other genes (peripheral) across the genetic background, *OsDEC* anchors a network that effectively mediates early transition to reproduction, thereby facilitating processes critical for grain productivity under drought. Transition of the meristem from vegetative to reproductive stage is mediated by cytokinin through the phospho-transfer system (*OsAHP1, OsPHP5*) leading to the enhancement of active cytokinin pools through the expression of the biosynthetic genes *OsLOGL3* and *OsLOGL7*, and concomitant suppression of *OsCKX2* involved in degradation. Induction of spikelet development is promoted by *OsMADS14*, *OsMADS15*, *OsMADS18*, and *OsMADS51*, and *OsHd3a* (florigen), which trigger the early onset of flowering under drought. Early formation of reproductive sink efficiently redirects the photosynthate to reproductive processes. The *taming* effect of ABA response (*OsLLB*, *OsABL1*) prevents unnecessary wastage of photosynthates that leads to large trade-offs to yield. C, control/irrigated; S, stress/drought; L, low-yield penalty backcross introgression line; H, high-yield penalty backcross introgression line; W, 'Way Rarem'; I, IR64; MeJA, methyl-jasmonate; ABA, abscisic acid; JA, jasmonate

suboptimal irrigation (Wu et al., 2019). It has also been shown that *OsMADS18* accelerates the transition of meristem from vegetative to reproductive by promoting the florigen *Hd3a* via cytokinin signaling (Fornara et al., 2004; Yoshida & Nagato, 2011). Methyl-jasmonate and abscisic acid (*ABA*) can cause significant reduction in yield through their direct impacts on reproductive structures (Davies et al., 2012; Kim et al., 2009). As such, the proper modulation of the pathway would be necessary to preserve yield, as depicted in the model (Figure 10). A functional *DEC*-network in LPB has the necessary components of a genetic machinery that could lead to enhanced pools of active cytokinins especially in flag leaves at the time of booting and during exposure to slow but progressive drought. Yield and yield-component data collected from the drought experiments performed for the transcriptomics studies recapitulated previously reported superior performance of LPB due to *qDTY12.1* effects (Figure 1).

4.4 | Potential modulation of ABA response in LPB based on transcriptome fluxes

Abscisic acid signaling is central to the first line of defense against drought but not without any costs to plant development and net productivity (Davies et al., 2012; Finkelstein & Rock, 2002; Kim et al., 2009; Tuteja, 2007; Zhang et al., 2006). Prioritization of cellular resources to balance the costs of survival with net productivity may require an extensive modulation of ABA responses. The overactive transcriptomic burst at booting stage in HPB, WR, and IR64 are indicative of a costly and "all in" response to drought, hence greatly perturbed cellular status. In contrast, the transcriptomic response in LPB at booting stage appeared to be more modulated or tamed (Figure 3). In other words, more is not necessarily always better as subtle changes could go a long way. Indeed, reports in other crops also showed much fewer number of differentially expressed genes in drought-tolerant genotypes compared to more sensitive genotypes (Bhogireddy et al., 2020; Fracasso et al., 2016). The overactive transcriptomic burst in HPB, WR, and IR64 based on the directionality of transcriptome fluxes may largely be associated with the ABA response.

A cursory evidence for the taming of the ABA response was illustrated by the differential expression of zeaxanthin epoxidase (ZEP; *Os04g0448900*) that catalyzes the first committed step in ABA biosynthesis via the xanthophyll cycle in plastids (Taylor et al., 2000; Tuteja, 2007; Verma et al., 2016). Drought-mediated upregulation or downregulation of ZEP was determined as a log2 fold-change from control values for each developmental stage (Supplemental Figure S8). A specific look at the booting stage showed a −0.57 log2 decrease in *ZEP* expression in LPB with drastic expression changes evident in HPB, WR, and IR64 at 4.1 log2 increase, 3.0 log2 decrease, and 1.9 log2 increase, respectively. Interestingly, inverse trends in *ZEP* expression across genotypes was evident at the vegetative and grain-filling stages. Drastic differences at booting stage suggest that LPB perhaps has the mechanism that fine-tunes ABA biosynthesis and therefore modulates the ABA response more efficiently. Based on the directionality of transcriptomic fluxes, it is apparent that the taming effects in LPB also extend beyond the genes involved in ABA responses.

5 | CONCLUSIONS

The *qDTY12.1*-encoded *OsDEC* represents the first candidate regulatory gene from any known drought-tolerance QTL in rice with potential direct links to yield-related cellular processes. The functionality of the *DEC*-network, or lack thereof, provides some explanation to the observed inconsistent effects of *qDTYs* across genetic backgrounds. Being the core of the network, coupling of a functional *OsDEC* allele with multiple positive-effect peripheral alleles across the background genome encoding for both the upstream and downstream components of its network, or in reverse, the uncoupling with multiple negative-effect peripheral alleles, could create either a fully functional, partially functional, or nonfunctional *DEC*-network. This dynamic is consistent with the Omnigenic theory. By virtue of the potential significance of *OsDEC* to cytokinin-mediated cellular processes, especially to the regulation of flowering time, we propose an important role of the *DEC*-network in facilitating the timely establishment of strong source-sink dynamics that sustains a robust reproductive transition during drought. The process uncovered in this study sets the foundation for further elucidation of molecular and biochemical mechanisms by forward or reverse genetics.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available as supplementary materials published online along with this paper. The complete set of transcriptomic data is available at the National Center for Biotechnology Information as PRJNA717284:SRR14074305-SRR14074328. Preprint of the manuscript has been deposited at Cold Spring Harbor Laboratory Preprint as bioRxiv doi: https://doi.org/10.1101/2021.02.09.430414.

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AUTHOR CONTRIBUTIONS

Jacobo Sanchez: Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft; Writing-review & editing. Pushpinder Pal Kaur: Formal analysis; Investigation; Methodology; Validation; Visualization. Isaiah C.M. Pabuayon: Formal analysis; Investigation; Methodology; Validation. Naga Bhushana Rao Karampudi: Formal analysis; Visualization. Ai Kitazumi: Formal analysis; Visualization. Nitika Sandhu: Investigation; Methodology; Resources. Margaret Catolos: Methodology. Arvind Kumar: Conceptualization; Methodology. Benildo G. de los Reyes: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Supervision; Validation; Writing-original draft; Writing-review & editing.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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