Received: 30 January 2024 | Revised: 29 July 2024 | Accepted: 16 August 2024

DOI: 10.1111/mec.17522

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Selection on genome-wide gene expression plasticity of rice in wet and dry field environments

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Funding information
Research Institute Centers, New York University Abu Dhabi; Graduate School of Arts and Sciences, Fordham University; University of California Riverside; Division of Environmental Biology, Grant/Award Number: DEB-1142784; Division of Integrative Organismal Systems, Grant/Award Number: IOS-1546218; Zegar Family Foundation; Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung, Grant/Award Number: P2BSP3_168833; National Institute of General Medical Sciences, Grant/Award Number: R35GM151194

Handling Editor: Clarisse Palma-Silva

Abstract

Gene expression can be highly plastic in response to environmental variation. However, we know little about how expression plasticity is shaped by natural selection and evolves in wild and domesticated species. We used genotypic selection analysis to characterize selection on drought-induced plasticity of over 7,500 leaf transcripts of 118 rice accessions (genotypes) from different environmental conditions grown in a field experiment. Gene expression plasticity was neutral for most gradually plastic transcripts, but transcripts with discrete patterns of expression showed stronger selection on expression plasticity. Whether plasticity was adaptive and co-gradient or maladaptive and counter-gradient varied among varietal groups. No transcripts that experienced selection for plasticity across environments showed selection against plasticity within environments, indicating a lack of evidence for costs of adaptive plasticity that may constrain its evolution. Selection on expression plasticity was influenced by degree of plasticity, transcript length and gene body methylation. We observed positive selection on plasticity of co-expression modules containing transcripts involved in photosynthesis, translation and responsiveness to abiotic stress. Taken together, these results indicate that patterns of selection on expression plasticity were context-dependent and likely associated with environmental conditions of varietal groups, but that the evolution of adaptive plasticity would likely not be constrained by opposing patterns of selection on plasticity within compared to across environments. These results offer a genome-wide view of patterns of selection and ecological constraints on gene expression plasticity and provide insights into

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Elena Hamann and Simon C. Groen equal contribution.

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the interplay between plastic and evolutionary responses to drought at the molecular level.

KEYWORDS
costs of adaptive plasticity, genotypic selection analysis, natural selection, *Oryza sativa* (rice), transcriptome profiling

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1 | INTRODUCTION

Many organisms have the ability to express trait plasticity, and this ability has long been recognized as potentially advantageous in response to spatial and temporal environmental variation (Alpert & Simms, 2002). A trait is considered plastic when the same genotype can express multiple phenotypes in response to environmental variation (Nicotra et al., 2010; Schlichting, 1986; Sultan, 1995; Via et al., 1995). This ability has been well studied in terrestrial plants, which are sessile and thus required to deal with ambient conditions and potentially multiple biotic and abiotic stresses while staying in place. Examples include inducible production of defensive compounds in response to herbivory (Agrawal et al., 2002; Groen et al., 2016) and morphological changes in stem elongation in response to shade (Dudley & Schmitt, 1995; Schmitt et al., 2003).

Plasticity of a phenotypic trait is often depicted as a reaction norm across varying environmental conditions (Schlichting, 1986; Schlichting & Pigliucci, 1998; Sultan, 1987). While plasticity can be studied as reaction norms across multiple environments, here we focus on plastic responses across two environments (Figure 1). When a genotype shows different trait values across environments, that genotype is phenotypically plastic for that trait, with the slope of the reaction norm indicating the degree of plasticity (Figure 1a). When plastic genotypes have the highest global fitness across environmental conditions, phenotypic plasticity would be adaptive (Figure 1b), yet plasticity is maladaptive when plasticity moves phenotypes away from their optima (Figure 1c) (Campbell-Staton et al., 2021; Ghalambor et al., 2007). Plastic responses may also be neutral, with little effect on fitness. If plasticity is favoured when fitness is averaged across environments (Figure 1d—thick line) and within environments (Figure 1e—thin line), then plasticity is adaptive and should evolve to increase for that trait (Byars et al., 2007; Ghalambor et al., 2007). However, if plasticity is favoured when fitness is averaged across environments (Figure 1d—thick line) but selected against within an environment (Figure 1e—thick line), then the evolution of plasticity may be constrained because of antagonistic directions of selection within compared with across environments, indicating a cost of adaptive plasticity (DeWitt et al., 1998; Murren et al., 2015). In this case, plasticity might be favoured when environments are highly variable in space or time, but selection against plasticity within a stable environment would reduce the overall benefit of plasticity and instead favour homeostasis (van Kleunen & Fischer, 2005, 2007).

As global change progresses and increases climate variability, phenotypic plasticity may play an important role in species' responses (Hamann et al., 2020; Jentsch et al., 2007). Yet, the interplay between plasticity and genetically-based evolutionary change remains a long-standing knowledge gap in the field of evolutionary genetics (Campbell-Staton et al., 2021; He et al., 2021; Nicotra et al., 2010). Recent models suggest plasticity could be advantageous and a critical first step towards adaptive evolution if associated costs are limited (Scheiner et al., 2020), but plasticity might also hamper species' evolutionary responses by weakening the strength of directional selection on trait values (Block et al., 2020; Oostra et al., 2018). The adaptive value of phenotypic plasticity depends on its direction relative to the phenotypic optimum (Campbell-Staton et al., 2021; Ghalambor et al., 2007, 2015; He et al., 2021; Price et al., 2003). As such, trait plasticity can be in the same or opposite direction as selection on mean trait values (co-gradient and adaptive, counter-gradient and maladaptive respectively) or neutral (Figure 1b,c). Plasticity can thus move individuals closer to (adaptive) or further away from (maladaptive) their phenotypic optima, which can in turn influence the strength and direction of selection on traits and evolutionary outcomes (Byars et al., 2007; Campbell-Staton et al., 2021). Additionally, plasticity itself may be under selection, and the evolution of plasticity may occur independently, or jointly with, changes in the mean trait value (Ghalambor et al., 2007).

The degree to which climatic and other environmental changes cause plastic adjustments, and the relative importance of plasticity compared to other responses such as migration or trait evolution, are still debated (Merila & Hendry, 2014). While plasticity is ubiquitous, the extent to which plasticity is adaptive and evolving under selection is incompletely understood (Arnold et al., 2019; Saltz et al., 2018; Sultan, 2004; Via et al., 1995). Several potential costs and limits of plasticity have been theorized as constraints to the evolution of plasticity (DeWitt et al., 1998), yet these concepts remain highly debated because empirical evidence for such constraints is rare (van Kleunen & Fischer, 2005). However, a review suggested that costs of plasticity are often biased or underestimated due to correlations between environment-specific trait values and the magnitude of trait plasticity (Auld et al., 2010) and that variable selection intensities or directions across environments may also impose strong constraints on the evolution of plasticity (Murren et al., 2015).

The plastic phenotypic response of an organism is largely mediated through regulation of the transcriptome, that is, plasticity of gene expression levels as molecular traits (Hodgins-Davis & Townsend, 2009). Plasticity can be assessed for any quantitative

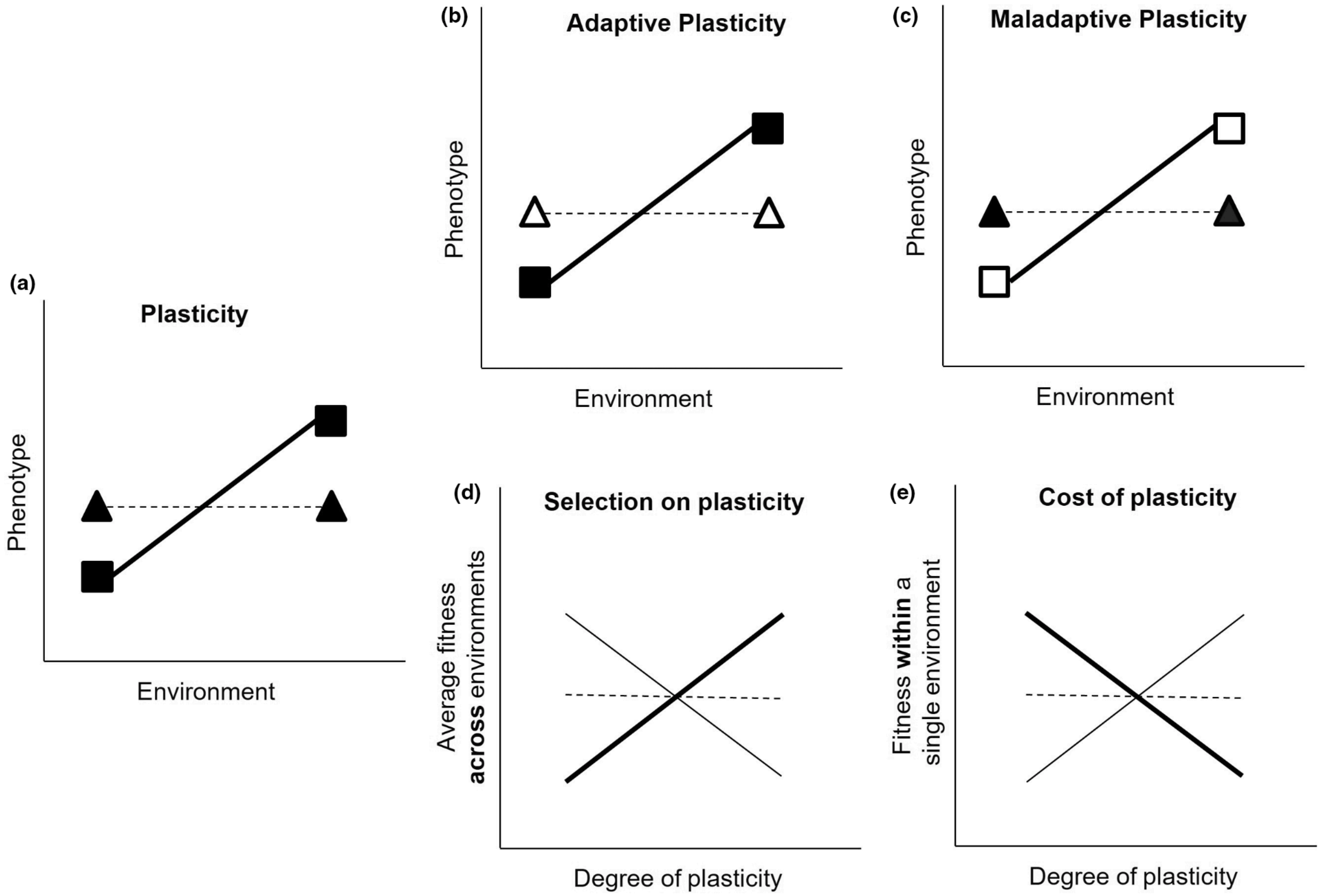

FIGURE 1 Reaction norms illustrating phenotypic plasticity and patterns of selection on plasticity within and across environments. In panel (a), the degree of plasticity of a genotype is represented as the slope of the reaction norm, with a plastic genotype (solid line) and a canalized (non-plastic) genotype (hatched line) shown. Panel (b) illustrates adaptive plasticity. Here, the dark, filled symbols represent the phenotype associated with greater fitness than the phenotype represented by the open symbols. Plasticity is adaptive and co-gradient when the more plastic genotype (solid line) has greater fitness in each environment than the non-plastic genotype, as shown. In contrast, when plastic genotypes have lower fitness in both environments (solid, thick line with open symbols) relative to non-plastic genotypes (hatched line with filled symbols) plasticity is maladaptive and counter-gradient (c). Panel (d) illustrates variation in selection on plasticity across environments. Here, plasticity might be favoured across environments and under positive selection (solid, thick line), disfavoured and under negative selection (selection for canalization) (solid, thin line), or neutral (dashed line). Panel (e) illustrates a cost of adaptive plasticity, which occurs when plasticity of a trait is favoured across environments (thick line in panel d) but is associated with decreasing fitness within an environment (solid, thick line). See text for details.

trait, but to date, most work has focused on higher-order traits such as growth or natural enemy defences (Agrawal et al., 2002; Groen et al., 2016; Relyea, 2002; Valladares et al., 2006; Van Buskirk & Steiner, 2009). Expression of a gene, however, is also a quantitative trait, and expression variation among isogenic individuals constitutes plasticity, which plays an integral role in adjustment of organismal physiology to environmental change (Schlichting & Smith, 2002). Genome-wide gene expression plasticity has been examined in response to various abiotic stresses, such as drought, heat, salinity and grazing, especially in crops and crop wild relatives, to improve breeding for sustainable agriculture (Dang et al., 2021; Priest et al., 2014; Zhou et al., 2007). Moreover, the transcriptome represents a direct link between genotype and phenotype, offering the possibility to examine the interplay between gene expression plasticity, fitness, and evolution (Ahmad et al., 2021; Groen et al., 2020; Koch & Guillaume, 2020). Because of advances in genomic technologies, it is now possible to measure genome-wide gene expression and fitness proxies for many individuals and to use a genomic reaction norm approach to study plasticity from a molecular perspective (Aubin-Horth & Renn, 2009; Oomen & Hutchings, 2022). It thus becomes feasible to assess gene expression plasticity, selection on plasticity, and whether plastic responses are co-gradient or counter-gradient with selection on gene expression (Figure 1). Similarly, we can examine the nature and extent of constraints or costs that could impede the evolution of plasticity (Figure 1). To our knowledge, this has never been attempted at the transcriptome level, although it could provide important information on how individuals cope with environmental variation from the genetic to phenotypic levels (Dayan et al., 2015; Kenkel & Matz, 2016; Mäkinen

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et al., 2015). We currently lack a detailed and comprehensive assessment of the interplay between gene expression plasticity and natural selection, as previous studies either considered plasticity of the transcriptome as a unified whole (Bittner et al., 2021; Dayan et al., 2015; Ghalambor et al., 2015; Kenkel & Matz, 2016; Mäkinen et al., 2015), considered specific candidate genes (Campbell-Staton et al., 2021; McCairns & Bernatchez, 2010), or measured selection on gene expression levels but not expression plasticity (Ahmad et al., 2021; Groen et al., 2020).

In this study, we examined selection on gene expression plasticity throughout the transcriptome in rice, *Oryza sativa* (L.). Rice is an important crop and genetic model system. Rice can be subdivided in two main varietal groups—Indica and Japonica—which we considered separately to account for population structure (Figure S1) (Choi et al., 2020; Groen et al., 2020; Wang et al., 2018). Traditional varieties are accessions of rice that have been grown for millennia across gradients of factors such as soil moisture and temperature (Gutaker et al., 2020). Our varietal groups contain mixtures of accessions that were sourced from four different rice agro-ecosystems that range from consistently wet to more intermittently dry (irrigated, deepwater, rainfed lowland and rainfed upland respectively) with the rainfed upland agro-ecosystem offering no standing water (flooding) around the base of the rice plants. Japonica varieties are predominantly grown in irrigated temperate lowlands and at higher altitudes in tropical and subtropical upland environments, whereas Indica varieties are mainly cultivated in irrigated and rainfed tropical and subtropical lowlands (Table S1). Relative fitness of Japonica versus Indica varieties in different environments frequently reflects their cultivation histories. For example, Japonica varieties tend to be more cold-tolerant than Indica varieties (Shakiba et al., 2017). Fluctuations within each of these environments occur and can be exacerbated by climate change, substantially influencing rice yields and fitness (Wing et al., 2018).

To examine selection on gene expression plasticity in rice, we conducted a large-scale field experiment in which Indica and Japonica accessions (mostly inbred traditional varieties) were subjected to dry or wet conditions, and measured how levels of gene expression affected plant fecundity, which is strongly correlated with grain yield, in each environment (Ćalić et al., 2022; Groen et al., 2020). We previously used this field experiment to assess selection on constitutive (non-plastic) gene expression. This prior study found widespread variation in gene expression, selection on expression for some transcripts, and differences in selection on gene expression patterns within the dry and wet environments separately (Groen et al., 2020). However, it did not determine if plasticity of expression was under selection, characterize patterns of selection on plasticity within and across environments, or evaluate whether potentially opposing selection patterns could constrain the evolution of expression plasticity. Thus, whether and to what extent expression plasticity is beneficial and adaptive in variable environments, or, whether its evolution is constrained under variable environments, remains unresolved for this system, as well as more broadly.

In the current study, we took advantage of this dataset (Groen et al., 2020) and built on our prior work by examining patterns of selection on gene expression plasticity. We determined how selection may act on drought-induced gene expression plasticity and how plasticity itself could affect selection on gene expression in rice using quantitative, population and systems genetics/genomics. Specifically, we examined: (1) heritability of gene expression plasticity, (2) selection on plasticity, (3) potential constraints on the evolution of plasticity due to opposing patterns of selection within relative to across environments (defined as costs of adaptive plasticity), (4) the relationship between selection on mean trait values and selection on plasticity, and specifically whether plasticity constrained (counter-gradient) or facilitated (co-gradient) selection on gene expression, (5) what genetic or metabolic factors impact patterns of selection on expression plasticity and (6) which biological processes may be influenced by selection on expression plasticity. One important aspect of our approach is that by using isogenic replicates within and across environments, we could calculate plasticity for each genotype, and then use fitness information to determine if there was selection for or against plasticity both within and across environments, while accounting for selection on mean trait values by including both plasticity and trait means in the statistical models. This allowed us to determine costs and benefits of plasticity itself, separate from costs of the phenotype produced by a plastic response (Murren et al., 2015). This approach provides insight into the evolutionary potential of gene expression and expression plasticity, as well as how plasticity and evolution can interact to determine responses to environmental changes at the genotypic and phenotypic levels.

2 | MATERIALS AND METHODS

2.1 | Data sources

We previously assessed transcriptome variation among 132 ‘Indica’ (indica and *circum*-aus) and 84 ‘Japonica’ (japonica and *circum*-basmati) accessions of rice (*Oryza sativa*) planted in a field experiment (Groen et al., 2020). In this experiment, conducted at the International Rice Research Institute in the Philippines, three individuals (genetically identical biological replicates) were planted per accession both in a continuously wet paddy field and a field that imposed intermittent drought (Groen et al., 2020). All experimental and analytical methods are described in detail in (Groen et al., 2020). Briefly, we used 3′-end mRNA sequencing (3′ mRNA-seq) to measure transcript levels in leaf blades of all plants at 50 days after sowing, 17 days after withholding water in the dry field, for a total of $n = 15{,}635$ widely expressed transcripts. After extraction, total RNA for each sample was processed individually according to a barcoded, plate-based 3′ mRNA-seq protocol (Satija Lab, 2018). Libraries of multiplexed sample pools were sequenced on the Illumina NextSeq 500, after which deconvoluted read data were quantified by aligning reads to the Nipponbare IRGSP 1.0 reference genome. For normalization, read

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counts were then scaled to transcripts per million, normalized using invariant-set normalization (Li & Wong, 2001), and converted to log space for further data analysis. Fecundity, defined as the number of filled grains, was measured destructively at season's end as a fitness proxy (Groen et al., 2020). There were 87 Indica accessions and 31 Japonica accessions that produced seeds and thus had a value of fecundity that could be used to approximate fitness, and these accessions (118 total) were included in the present study.

2.2 | Genotype × environment interaction

For each transcript, we fit a linear mixed model partitioning variance between the terms 'genotype' (G, random factor), 'environment' (E, fixed factor), G × E interaction (random factor), and error variance (ε) using the package *lme4* in *R* v3.6.3 (R Core Team, 2017).

2.3 | Source of plasticity

Significant G × E interaction may come from two sources: deviation of the cross-environment genetic correlation (r_{GE}) from unity, and differences in the among-genotype variance between environments (Gutteling et al., 2007). Cross-environment genetic correlations were estimated as $r_{GE} = cov_{ij}/\sigma_i\sigma_j$, where cov_{ij} is covariance of accession means between a transcript's expression level as *i* in wet and *j* in dry conditions, and σ_i and σ_j are the square roots of among-genotype variance for the transcript in wet and dry conditions. We determined the contribution from each source using: $V_{G\times E} = 0.5(\sigma_i - \sigma_j)^2 + \sigma_i\sigma_j(1 - r_{GE})$, where $V_{G\times E}$ is $G \times E$ variance, σ_i and σ_j are the square roots of among-genotype variance for the transcript's expression level in wet and dry conditions (Gutteling et al., 2007).

2.4 | Heritability

We estimated broad-sense heritability for each transcript by estimating variance explained by genotype within each environment as $H^2 = \sigma^2{}_G / \left(\sigma^2{}_G + \sigma^2{}_E\right)$ where $\sigma^2{}_G$ is among-genotype variance and $\sigma^2{}_E$ error variance (West et al., 2007). While broad-sense heritability does not differentiate between additive and non-additive genetic variance (i.e. dominance or epistasis), this estimate reflects the portion of phenotypic variance attributable to genetic causes, potentially under selection, for these inbred rice accessions (Konate et al., 2016; Roy & Shil, 2020).

2.5 | Differential gene expression

We used the Bioconductor package *limma* to identify transcripts that were differentially expressed between environments for each accession (Gentleman et al., 2004; Ritchie et al., 2015). *Limma* employs an empirical Bayesian approach for identifying differentially expressed transcripts. This approach models transcript expression data under the assumption that variation in expression is normally distributed, with prior probabilities for differential expression set as equal for all transcripts. These assumptions are not always biologically realistic. Furthermore, the ability of *limma* to detect differential expression gradually decreases at increasing expression ratios, particularly if these ratios exceed a $\log_2$ fold change of 1.5 (Rapaport et al., 2013). We took several steps to address these limitations. We applied invariant set normalization (Li & Wong, 2001) to our expression data, which helps make it more likely that the assumption of normal distribution of expression values is met. We also employed a minimal $\log_2$ fold change threshold of 1, using the false discovery rate (FDR) at $q < 0.05$ to control for false positives (Benjamini & Hochberg, 1995). Accession-level variation in expression plasticity was quantified as the number of accessions with significant drought-modified expression for each transcript (Des Marais et al., 2012).

2.6 | Plasticity quantification

Based on how widespread transcripts were expressed across genotypes, individual replicates, and wet and dry environments, we distinguished two sets of transcripts for the current study: (1) gradually plastic transcripts (GPTs) with continuous expression levels across environments and (2) discretely plastic transcripts (DPTs) that are more frequently switched on or off, with environment-specific expression (Stearns, 1989). For GPTs, we selected widespread transcripts as expressed by at least two of the three individual replicates in >75% of genotypes in a varietal group in each of the two environments. DPTs had to show the same widespread expression, but in only one environment (since they are only expressed in one environment, by definition). The selection criteria mirror the methods used for previous analyses of selection on baseline gene expression levels (Groen et al., 2020).

For GPTs and DPTs, we calculated absolute values of plasticity metrics to avoid biases regarding transcripts' biological roles; for example, transcription factors can be activators or repressors (Wilkins et al., 2016). GPTs can be regarded as quantitative functional traits, and we quantified the plasticity of transcript expression across environments for each genotype as the simplified relative distance plasticity index ($\mathrm{RDPI_s}$), which allows for statistical comparisons of genotypes (Valladares et al., 2006). We calculated $\mathrm{RDPI_s}$ as the absolute difference of mean genotypic transcript levels across environments divided by the mean genotypic transcript level in the wet environment, following Valladares et al. (2006), with $\mathrm{RDPI_s}$ as $P_j = |Z_{j,k=2} - Z_{j,k=1}|/Z_{j,k=1}$, where *j* is Genotype, *k* Focal environment, *Z* Transcript value, and *P* Transcript plasticity.

For DPTs, whose expression is null in one of the two environments, we calculated the coefficient of variation over the environments based on means ($\mathrm{CV_m}$) as in Schlichting and Levin (1984) and Schlichting (1986), a measure strongly correlated with $\mathrm{RDPI_s}$ (Valladares et al., 2006).

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2.7 | Genotypic selection analyses

Phenotypic selection analysis examines patterns of selection by regressing trait values against fitness (Lande & Arnold, 1983), while genotypic selection analysis uses a similar regression approach but with family means or other estimates of genotypic trait values to avoid biases inherent in phenotypic selection analysis (Rausher, 1992). We conducted genotypic selection analyses, which were based on gene expression values and relative fecundity fitness (filled-grain number), averaged across replicate individuals of each genotype. Genotypic selection analyses were conducted with genotypic fitness values averaged across environments to assess the strength and direction of selection on gene expression plasticity (i.e. whether plasticity is adaptive and selected for across variable environments), as well as within the wet and dry environments to assess potential costs of adaptive plasticity (Arnold et al., 2019; DeWitt et al., 1998; Murren et al., 2015; Relyea, 2002; van Kleunen & Fischer, 2007), as explained below.

We calculated relative fitness for each genotype by dividing its average filled-grain number by the mean of all genotypes across both environments. Mean transcript expression values were then standardized for all genotypes (mean = 0, SD = 1) across environments. Similarly, the $\mathrm{RDPI_s}$ and $\mathrm{CV_m}$ values calculated for transcript level plasticity across environments were standardized for all genotypes (mean = 0, SD = 1). Furthermore, genotypes that were severe outliers for the relative abundance of a transcript (±3 SD) were removed on a per-transcript basis to satisfy the assumption of normality for the selection analyses as was done for previous analyses of selection on baseline gene expression levels (Groen et al., 2020).

To examine selection on constitutive transcript expression (selection on baseline expression level rather than on change in expression), we estimated the total linear selection differential S as the regression coefficient of relative fitness on the standardized mean expression level (Lande & Arnold, 1983). To additionally examine selection on transcript plasticity (selection on the degree to which a genotype changes in expression across environments), we estimated β as the partial regression coefficient of relative fitness on the standardized mean transcript level value and transcript plasticity index (Relyea, 2002). We focused on β because this value is directly proportional to the response to selection, which is a measure of microevolution and thus indicates whether plasticity itself may evolve (Hendry & Kinnison, 1999; Lande & Arnold, 1983). A positive partial regression coefficient of relative fitness on the plasticity index indicates that plasticity is selected for and adaptive, while a negative coefficient indicates that plasticity is selected against and maladaptive. Because the model includes both plasticity and mean trait values, the partial regression coefficient for the plasticity index reflects selection on plasticity itself, apart from selection on the phenotypic value produced by the plasticity (Murren et al., 2015; Relyea, 2002).

We conducted an analysis to determine if the evolution of plasticity in gene expression might be constrained by opposing patterns of selection within compared to across environments (Figure 1e; Relyea, 2002; Auld et al., 2010; Murren et al., 2015). This analysis took advantage of the fact that our experiment included replicated genotypes planted in each environment, allowing us to calculate selection on plasticity of each genotype both within and across environments. For this analysis, we used relative fitness calculated as the mean genotypic filled-grain number produced in one environment (wet or dry) divided by the average filled-grain number of all genotypes in that same environment. Similarly, transcript expression values within each environment as well as $\mathrm{RDPI_s}$ and $\mathrm{CV_m}$ values were standardized for all genotypes (mean = 0, SD = 1). We assessed a cost of adaptive plasticity (DeWitt et al., 1998) as cases in which there is selection favouring plasticity when fitness is measure across environments and selection against plasticity within an environment (Figure 1d,e). To do so, we selected transcripts that showed significant positive selection on plasticity when considering fitness averaged across environments ($\mathrm{RDPI_s}$ and $\mathrm{CV_m}$ β at $\alpha = 0.01$), and then for these transcripts, we looked for cases in which there was selection against plasticity when considering fitness within an environment ($\mathrm{RDPI_s}$ and $\mathrm{CV_m}$ β at $\alpha = 0.01$). Such a contrasting effect of plasticity on fitness across environments compared to within environments constitutes evidence for a cost of adaptive plasticity, which could constraint the evolution of plasticity (DeWitt et al., 1998; van Kleunen & Fischer, 2005). A relatively conservative 1% α-threshold was used to account for the regressions performed on thousands of transcripts.

All genotypic selection analyses were performed in *R* v3.6.3 (R Core Team, 2017). Regression outputs were retrieved using the *broom* package in the *tidyverse* modelling set (Wickham et al., 2019), and we extracted selection differentials for constitutive transcript expression (S), transcript plasticity (β) and their corresponding statistical significance levels (Relyea, 2002). Additionally, we assessed our models for potential biases by calculating the Variance Inflation Factor (VIF), using the *car* package, to determine if there were correlations between trait values and the magnitude of plasticity that would lead to multicollinearity and confound selection on trait values with selection on trait plasticities (Auld et al., 2010).

To assess co- or counter-gradient selection (Byars et al., 2007) for both the GPTs and DPTs, we categorized the transcripts based on whether they showed a significant effect of environment ($p < 0.01$) as determined by linear mixed model fitting and whether differential expression across environments was manifested as up- or down-regulation in the dry versus the wet environment. We further categorized transcripts by whether selection on constitutive transcript levels within the wet and dry environments was under positive or negative directional selection (S at $\alpha = 0.01$). When differential expression and selection on constitutive expression were in the same direction, selection on expression was considered to be co-gradient. When expression and selection on constitutive expression were in opposite directions, selection on expression was considered counter-gradient. We tested whether co- or counter-gradient patterns occurred significantly more or less frequently than expected by chance using χ^2 tests.

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2.8 | Factors affecting selection on gene expression plasticity

For both types of plastic transcripts, we assessed whether average selection (β) on expression plasticity was influenced by factors related to metabolic, gene architectural and network features. Specifically, we focused on gene characteristics previously found to influence evolutionary change in gene expression in this system, such as r_{GE} and heritability, primary transcript length, GC content, tissue-specificity (tau), expression polymorphism and noise, mean expression level, and transcript connectivity (Groen et al., 2020). We also examined characteristics known to influence gene expression, including gene body methylation (gbM), transcript stability and translatability, the length and GC content of UTRs and CDSs (Chodavarapu et al., 2012; Elhaik et al., 2014; Su et al., 2018; Zhao et al., 2017), the number of introns per gene (https://plants.ensembl.org/biomart/), and codon usage (Nakamura et al., 2000). Based on codon usage, we calculated the total and relative (to molecule length) number of N atoms per transcript (Kelly, 2018), and C and N atoms per protein (Arnold & Nikoloski, 2014). We also calculated ATP expenditure per transcript using general eukaryote-derived values (Lynch & Marinov, 2015), and per encoded protein using plant-derived values from Arabidopsis, ignoring the negligible protein assembly costs (Arnold & Nikoloski, 2014).

We included these 25 covariates alongside β in partial correlation analyses ($n=3,772$ and $n=3,058$ for GPTs and DPTs in Indica, respectively, while $n=3,789$ and $n=3,580$ for GPTs and DPTs in Japonica, respectively) using the R package CORPCOR version 1.6.9 (R Core Team, 2017; Schäfer & Strimmer, 2005). We first calculated Pearson product–moment correlations between pairs of all variables, before estimating the partial correlations by establishing the pseudo-inverse of the resulting correlation matrices (Larracuente et al., 2008). Results were visualized in heatmap format with *ClustVis* (Metsalu & Vilo, 2015).

2.9 | Co-expression analysis

To infer the biological relevance of selection on gene expression plasticity, we filtered GPTs for significant heritability of plasticity (i.e. a significant G×E term, $p<0.05$) and retained 1045 and 494 transcripts for Indica and Japonica, respectively. We then conducted *k*-means clustering analyses in iDEP to infer modules of co-expressed genes in an unsupervised manner using a jack-knifing approach to determine module number (Ge et al., 2020). We inferred separate networks of heritably plastic, co-expressed transcripts in Indica and Japonica. We did not perform this analysis for DPTs, since the on/off expression patterns reduce the power for identifying biologically meaningful modules of co-expressed transcripts.

2.10 | Gene-set enrichment analysis

For transcript modules with evidence of selection on gene expression plasticity levels, we examined gene functional annotations. We considered gene ontology (GO) biological processes, using PANTHER's Overrepresentation Test (released February 24, 2021) with the *O. sativa* genes in the GO database (DOI: 10.5281/zenodo.4495804; released 1 February 2021) as background gene set used to match the foreground set (Mi et al., 2020). A Fisher's exact test was applied to identify the most enriched GO biological processes.

3 | RESULTS

3.1 | Patterns of selection on transcript plasticity

We found evidence for genotype-by-environment interaction (G×E) in a portion of transcripts examined, indicating that expression level differed between dry and wet conditions for these genotypes. Specifically, of the 15,635 transcripts examined, after FDR correction for multiple tests, ~17.3% showed significant G×E among Indica accessions (Figure 2; Table S2). This proportion was lower (~8.9%) among Japonica accessions (Figure S2; Table S2). The transcripts that showed significant G×E tended to exhibit higher heritability within each of the environments (median $H^2_{\text{Wet}}=0.58$ and $H^2_{\text{Dry}}=0.57$ respectively) than transcripts that did not show significant G×E (median $H^2_{\text{Wet}}=0.51$ and $H^2_{\text{Dry}}=0.50$ respectively) for Indica accessions (Figure 2; Table S2), with similar patterns observed for Japonica accessions (Figure S2; Table S3). In keeping with significant G×E for many transcripts, we observed that most drought-induced transcript-level changes occurred in only one or a few genotypes (Figure 2; Figure S2; Table S4). For both varietal groups, G×E was mostly due to differences in the among-genotype variance in gene expression levels between environments (Figure 2; Figure S2; Table S5).

Known drought-responsive transcripts were among the most plastic. Specifically, the set of the top ~5% of transcripts that were most frequently differentially expressed between wet and dry environments was enriched for several cellular metabolism-related GO biological processes, as well as responses to external stimuli and cell morphogenesis (Figure S3; Table S6).

Transcripts were grouped into GPTs or DPTs depending on their expression patterns across environments. Inclusion of transcripts within one or the other category was not influenced by general patterns of presence/absence variation (PAV) of genes in *O. sativa* (Figure S4; Table S7). We analysed 3,772 GPTs and 3,058 DPTs for Indica accessions, as well as 3,789 GPTs and 3,580 DPTs for Japonica accessions (Table S8, S9). Plasticity indices used to quantify plasticity for GPTs and DPTs (RDPI_s and CV_m respectively) were highly correlated for both varietal groups (Figure S5). We estimated selection differentials for each GPT expressed among the Indica and Japonica varietal groups separately (Tables S10 and S11), and our model verifications detected no multicollinearity issues (VIF <3 for GPTs). Plasticity was neutral for most transcripts, but for those transcripts showing selection on plasticity, plasticity was more often selected for than against. In Indica, selection on expression plasticity appeared to be relatively weak: the median selection differential for plasticity was $|\beta|_{\text{median(Ind)}}=0.0291$, with

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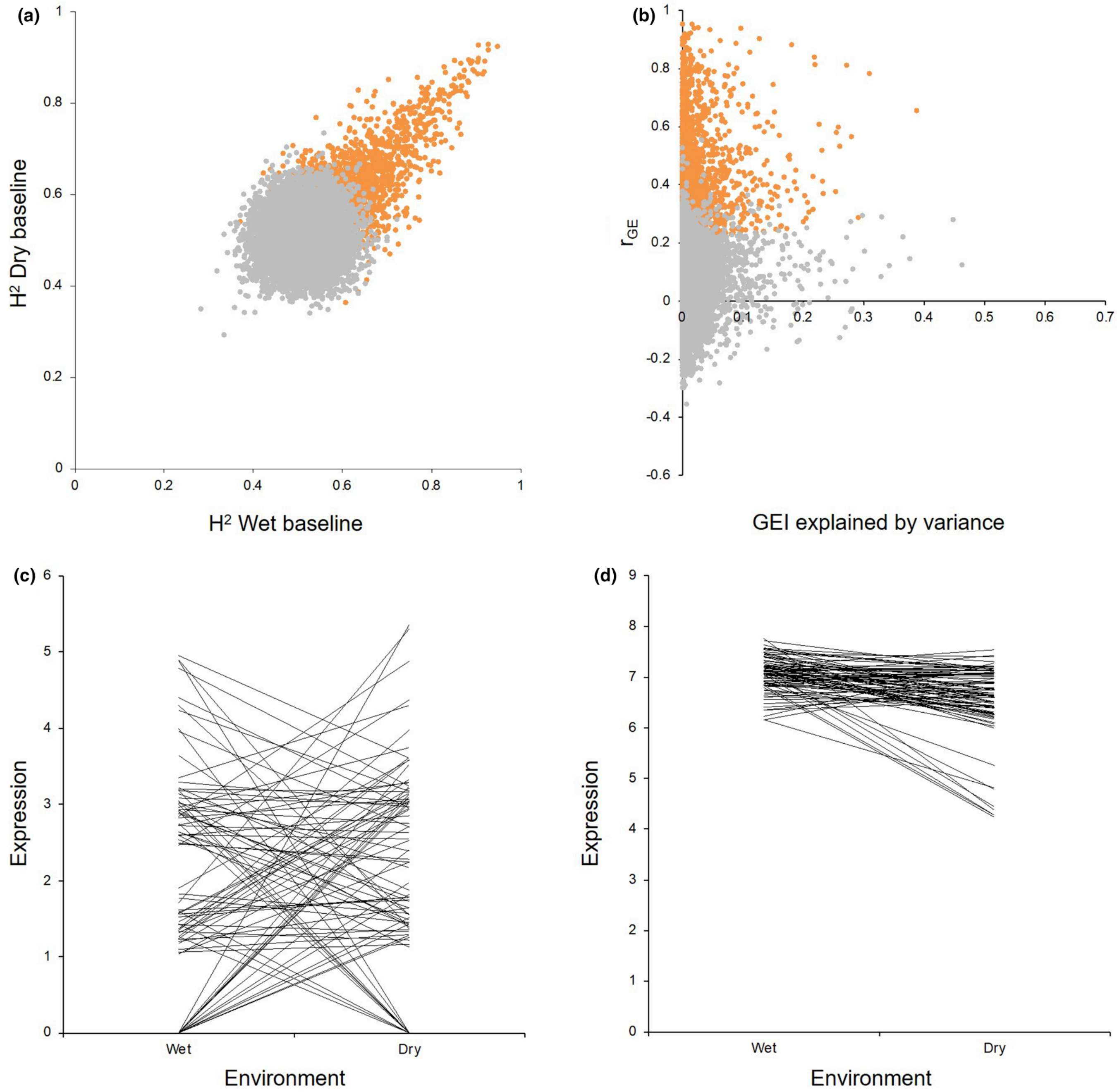

FIGURE 2 Bivariate plot of broad-sense heritability (H^2) estimates for constitutive transcript expression levels in the Indica populations in wet and dry conditions (a). Orange dots indicate significant transcript G × E interaction variance (FDR q value <0.001), and grey non-significant. Proportion of G × E interaction variance attributed to changes in among-accession transcript variance in wet and dry conditions versus r_{GE}. Orange dots indicate significant transcript G × E interaction variance (FDR q value <0.001), and grey non-significant (b). Reaction norms of a transcript for which G × E interaction variance is mostly determined by deviation of r_{GE} from unity, as indicated by abundant line crossing (c). Reaction norms of a transcript for which more G × E interaction variance is determined by changes in among-accession variance in wet and dry conditions, as indicated by less-abundant line crossing and wider among-accession variance in one environment than the other (d).

only ~2.5% of transcripts showing $|\beta| > 0.1$ (Figure 3a; Table S10), suggesting that—for most genes—variation in expression plasticity is neutral. However, there was slightly stronger selection for ($\beta_{\text{median(pos)}} = 0.0302$) rather than against ($\beta_{\text{median(neg)}} = -0.028$) plasticity in Indica (Mann–Whitney *U*-test [MWt], one-tailed $p = 0.0401$; Figure 3b). In Japonica, a similar pattern was visible, although selection on plasticity was generally stronger with $|\beta|_{\text{median(Jap)}} = 0.1075$ (MWt, $p < 0.0001$; Figure S6a; Table S11). A trend towards stronger selection for rather than against plasticity was also visible in Japonica with $\beta_{\text{median(pos)}} = 0.1342$ and $\beta_{\text{median(neg)}} = -0.1241$, respectively, but this difference was not significant (MWt, $p = 0.484$; Figure S6b).

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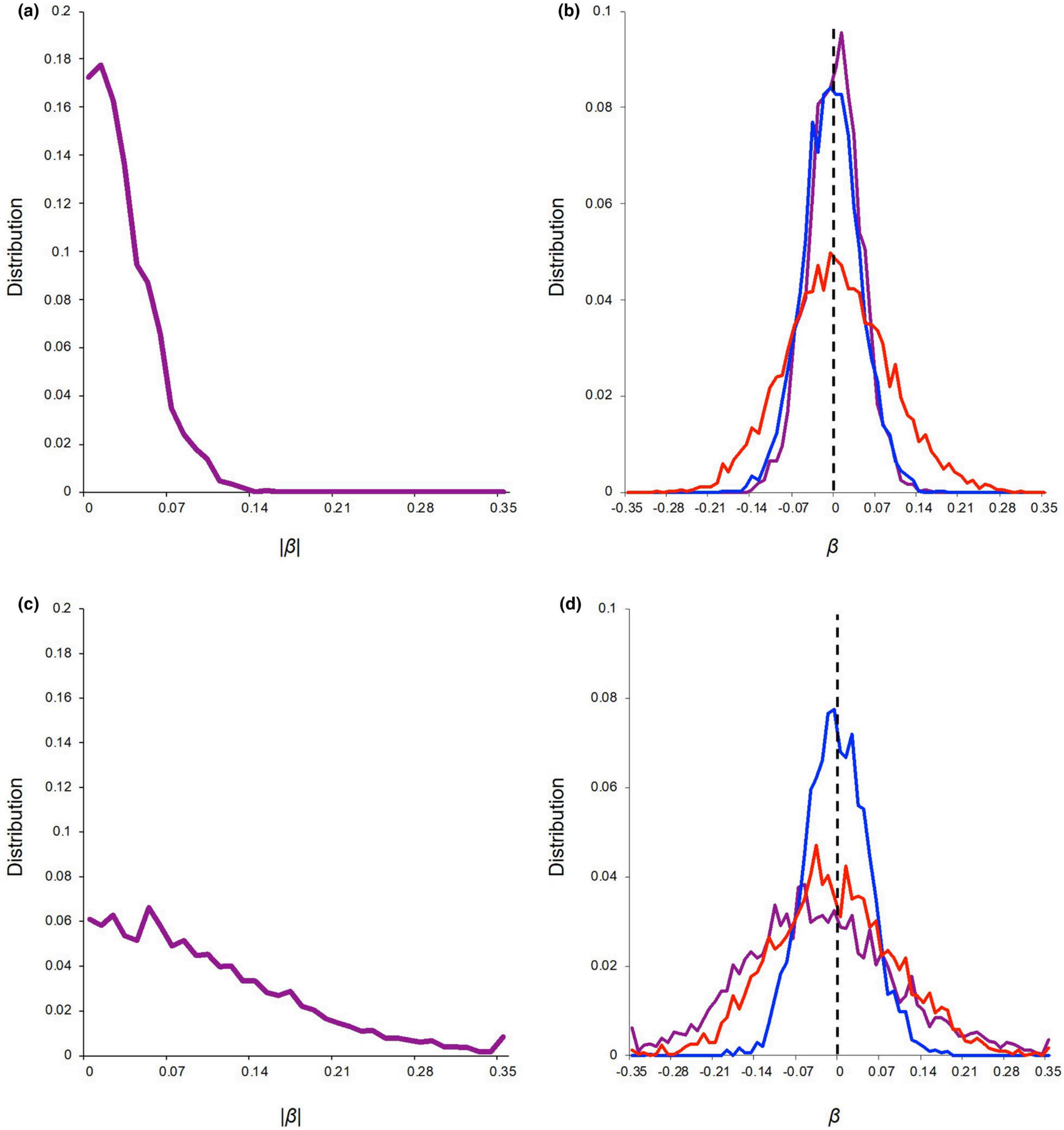

FIGURE 3 Patterns of selection $|\beta|$ on gene expression plasticity in Indica for gradually plastic transcripts (GPTs; a and b) and for discretely plastic transcripts (DPTs; c and d) using fecundity as a fitness measure. For GPTs, selection was relatively weak for most transcripts ($|\beta| < 0.1$) (a); positive directional selection on transcript level plasticity was stronger than negative directional selection across environments (purple), and selection differential values were generally higher in dry (red) than in wet (blue) conditions with ($|\beta|_{\text{median(dry)}} = 0.0581$) and ($|\beta|_{\text{median(wet)}} = 0.032$) respectively (MWt, $p < 0.0001$; b). For DPTs, a substantial number of transcripts showed relatively strong selection ($|\beta| > 0.1$) (c); negative directional selection on transcript level plasticity was stronger than positive directional selection across environments (purple), and selection differential values were generally higher in dry (red) than in wet (blue) conditions with ($|\beta|_{\text{median(wet)}} = 0.036$) and ($|\beta|_{\text{median(dry)}} = 0.069$) respectively (MWt, $p < 0.0001$; d).

For DPTs, we detected collinearity (VIF > 10 for the majority of transcripts) between the baseline expression values and the plasticity index (CV_m). This correlation is inherent to the fact that transcripts are only expressed in one of the two environments. Thus, the magnitude of plasticity is necessarily correlated with expression values. While there is no immediate analytical solution to this potential bias

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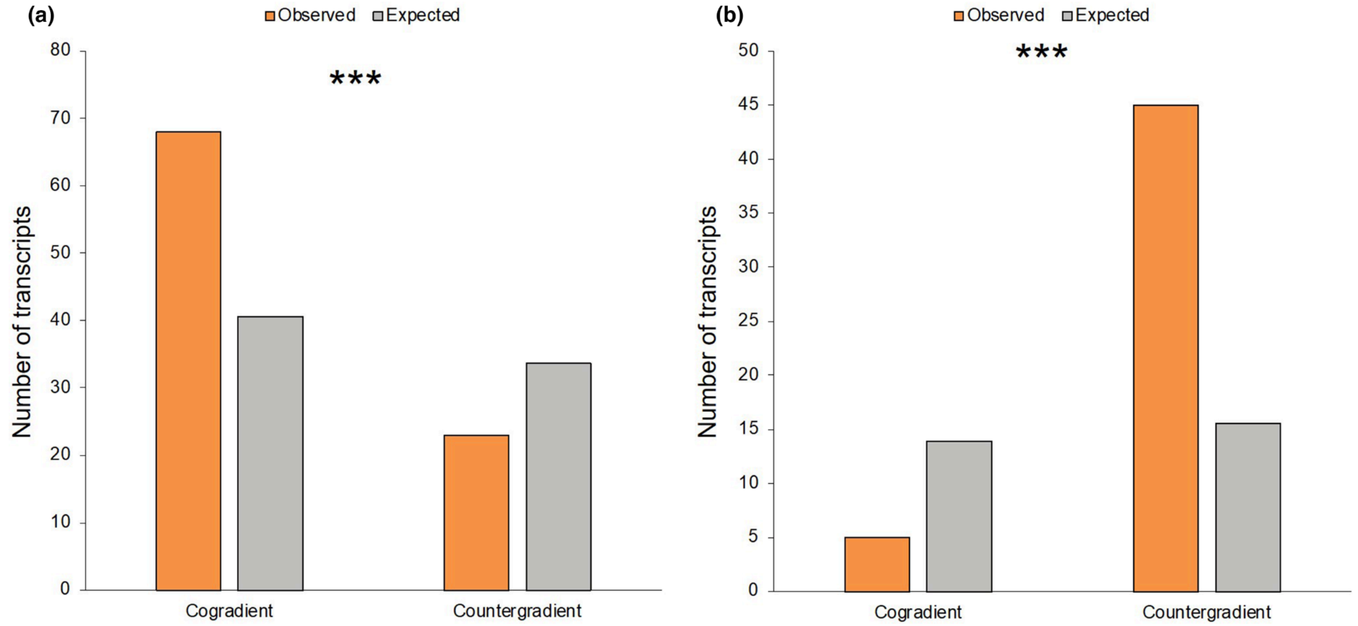

FIGURE 4 Co-gradient and counter-gradient selection. In Indica, the direction of selection on constitutive expression in wet and dry conditions (*s*) was co-gradient with the direction of expression plasticity more frequently than expected by chance (a). In Japonica, the direction of selection on constitutive expression in wet and dry conditions (*S*) was counter-gradient with the direction of expression plasticity more frequently than expected by chance (b). Asterisks (***) indicate $p < 0.001$.

because both variables are needed in the models to quantify the effects of plasticity while controlling for the expression profiles (Auld et al., 2010), we acknowledge that selection differentials across environments for DPTs should be interpreted with caution. Plasticity of DPTs was overall under stronger selection than that of GPTs in Indica, with $|\beta|_{\text{median(graded)}} = 0.0291$ and $|\beta|_{\text{median(discrete)}} = 0.0879$, respectively (MWt, $z = -42.16$, $p < 0.0001$; Figure 3c; Table S10), indicating that the population distribution of plasticity levels for drought-responsive DPTs was further removed from the phenotypic optimum. In contrast to GPTs, plasticity in DPTs was generally selected against, meaning that canalization was beneficial. This can be seen by the fact that for DPTs, β showed an overall bias for stronger negative than positive values, with $\beta_{\text{median(pos)}} = 0.0746$ and $\beta_{\text{median(neg)}} = -0.0973$, respectively (MWt, $p < 0.0001$; Figure 3d; Table S10). Also, in Japonica, selection on plasticity was generally stronger for DPTs than GPTs (Figure S6c,d; Table S11), indicating that the expression distributions for DPTs were overall further from adaptive peaks.

3.2 | Potential constraints on the evolution of transcript plasticity

Transcripts in which plasticity was under positive selection across environments (β_{across} at $\alpha = 0.01$) give evidence for adaptive plasticity. We found that for GPTs, plasticity was selected for and would be adaptive for 63% of transcripts in Indica and 91% in Japonica based on selection for their levels of plasticity across environments ($\alpha = 0.01$) (Figure S7; Tables S10 and S11). For DPTs, transcript plasticity across environments was selected for in 33% of transcripts in Indica and in 38% of transcripts in Japonica (Figure S7; Tables S10 and S11).

To test for costs of adaptive plasticity, we examined if there were transcripts in which plasticity was selected for across environments (β_{across} positive) but against within environments (β_{wet} and/or β_{dry} is negative). These are transcripts from the positive and negative tails of the distributions (Figure 3b,d; Figure S6b,d). Transcripts with positive β_{across} never overlapped with transcripts showing negative β_{wet} or β_{dry}, indicating that this type of cost of adaptive plasticity was not detected in either the Indica or the Japonica varietal groups (Figure S7; Tables S10 and S11).

3.3 | Effects of plasticity on selection

We investigated whether directional selection on constitutive levels of transcript expression was co-gradient or counter-gradient with the direction of expression plasticity to determine if plasticity enhanced or constrained responses to selection on expression. In Indica, the direction of gene expression plasticity for GPTs and DPTs was more frequently co-gradient with the direction of *S* on transcript expression than expected by chance, whereas a counter-gradient pattern occurred less frequently ($\chi^2 = 21.832$, $p = 2.977 \times 10^{-6}$; Figure 4a; Table S12). Thus, plasticity does not appear to counter selection on expression in Indica and may have contributed to evolutionary responses to selection. However, this pattern was reversed in Japonica, with directions of expression plasticity more frequently than expected by chance running counter-gradient with directional selection on expression

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($\chi^2 = 61.775$, $p = 3.849 \times 10^{-15}$; Figure 4b; Table S12), indicating that plasticity may constrain the evolutionary response to selection on expression plasticity in Japonica. Thus, the degree to which plasticity in expression enhanced or constrained responses to selection on expression varied among varietal groups rather than showing a consistent pattern.

3.4 | Factors affecting selection on gene expression plasticity

We examined whether the strength and pattern of selection on gene expression plasticity was linked to factors related to constitutive gene expression, gene architecture, metabolic costs and network features. In Indica, β_{across} showed significant negative partial correlations with transcript length for GPTs as well as with gene body methylation (gbM) for GPTs and DPTs ($p \leq 0.0002$; Figure 5a,b), which are both factors related to gene architecture and metabolic costs. We did not detect any significant relationships between selection on expression plasticity and factors related to gene network features (Tables S13 and S14).

3.5 | The biological role of transcripts experiencing selection on gene expression plasticity

Because genes may correlate in their expression patterns, and selection may act on variation in levels of a multitude of transcripts simultaneously (Hämälä et al., 2019; Huang et al., 2020), we also examined selection on co-expression modules for GPTs. We excluded DPTs from this analysis because of their much lower levels of connectivity than GPTs (0.93× difference, $p = 7.24 \times 10^{-36}$; Figure S4) based on measurements over a time series of the tightness of their co-expression with other transcripts (Plessis et al., 2015), which was due to their discontinuous expression across environments. We focused on transcripts that showed significant G×E, since selection on the plasticity of these transcripts has the potential to cause evolutionary changes in plasticity (Hendry & Kinnison, 1999).

For Indica, the 1045 transcripts retained for network analysis after filtering for heritability of expression plasticity could be grouped into 13 modules of co-expressed transcripts (Figure 6a; Table S1). Modules B to I showed significant positive selection on expression plasticity whereas the other modules did not (Figure 6b). The set of transcripts in these modules was enriched in GO biological processes related to photosynthesis, translation and responsiveness to abiotic stress (Figure 6c), suggesting selection on environment-induced plasticity of the molecular machinery regulating these biological processes. There was no selection against plasticity for these modules in either wet or dry conditions (mean $\beta_{\text{wet}} = 0.0138$; mean $\beta_{\text{dry}} = 0.0121$).

In Japonica, two modules showed significantly negative β values overall (Figure S8; Table S15). These modules under negative selection were enriched in translation-related transcripts (Figure S8), suggesting opposite patterns of selection on the expression plasticity of such genes between the Indica and Japonica accessions.

Finally, we examined whether factors related to metabolic and gene architectural features could suggest a biological explanation for patterns of negative selection on expression plasticity of DPTs (Figure 3d; Figure S6d). As previously described, plasticity of DPTs was more frequently selected against than for, and expression of the underlying genes was more frequently shut down rather than activated under drought stress conditions. This latter pattern seemed to be associated with certain gene and transcript architectural features. Compared to GPTs, DPTs exhibited wider potential expression across tissues as indicated by a 0.93× difference in the tissue specificity index τ ($p = 3.29 \times 10{-}24$; Figure 7a), and DPTs were longer than GPTs (1.19× difference, $p = 1.99 \times 10{-}41$; Figure 7a; Table S7). Promoters of the underlying genes contained fewer TF binding sites (median of 5 instead of the of 6 regulatory elements for GPTs, $p < 0.0001$; Figure 7b; Table S7), and TATA-boxes (23.4% vs. 25.7%, $p = 0.002$; Figure 7c; Table S7). Furthermore, gbM levels were higher for the less abundant DPTs than for the more abundant GPTs (1.25× difference, $p = 8.12 \times 10{-}11$; Figure 7a). All of these patterns were similar for Japonica (Figure S9; Table S7).

4 | DISCUSSION

Our study provides novel insights into factors influencing the benefits of gene expression variation and potential constraints on its evolution by analysing selection on the plasticity of expression of over 7500 genes in rice subjected to dry or wet conditions in the field. We found selection on plasticity in gene expression in response to drought in a small fraction of transcripts examined. Some of these transcripts had functions related to drought stress responses, and there was selection for plasticity of gene modules linked to photosynthesis- and translation-related genes. Whether plasticity of expression was co- or counter-gradient with selection on gene expression depended on the rice varietal group, indicating that the effects of plasticity on the evolution of gene expression could vary among locations and selective pressures. Selection on plasticity of expression was related to gene architectural features. We found no cases in which there was selection favouring plasticity across environments and selection against plasticity within an environment, indicating the absence of evidence for this type of cost of adaptive plasticity. These results help us understand the role that variation in plasticity of gene expression can play in evolutionary responses to changing climatic conditions.

4.1 | Selection and constraints on gene expression plasticity

Plasticity can evolve when there is significant G×E interaction (Schlichting & Pigliucci, 1998). Yet, environment-specific expression

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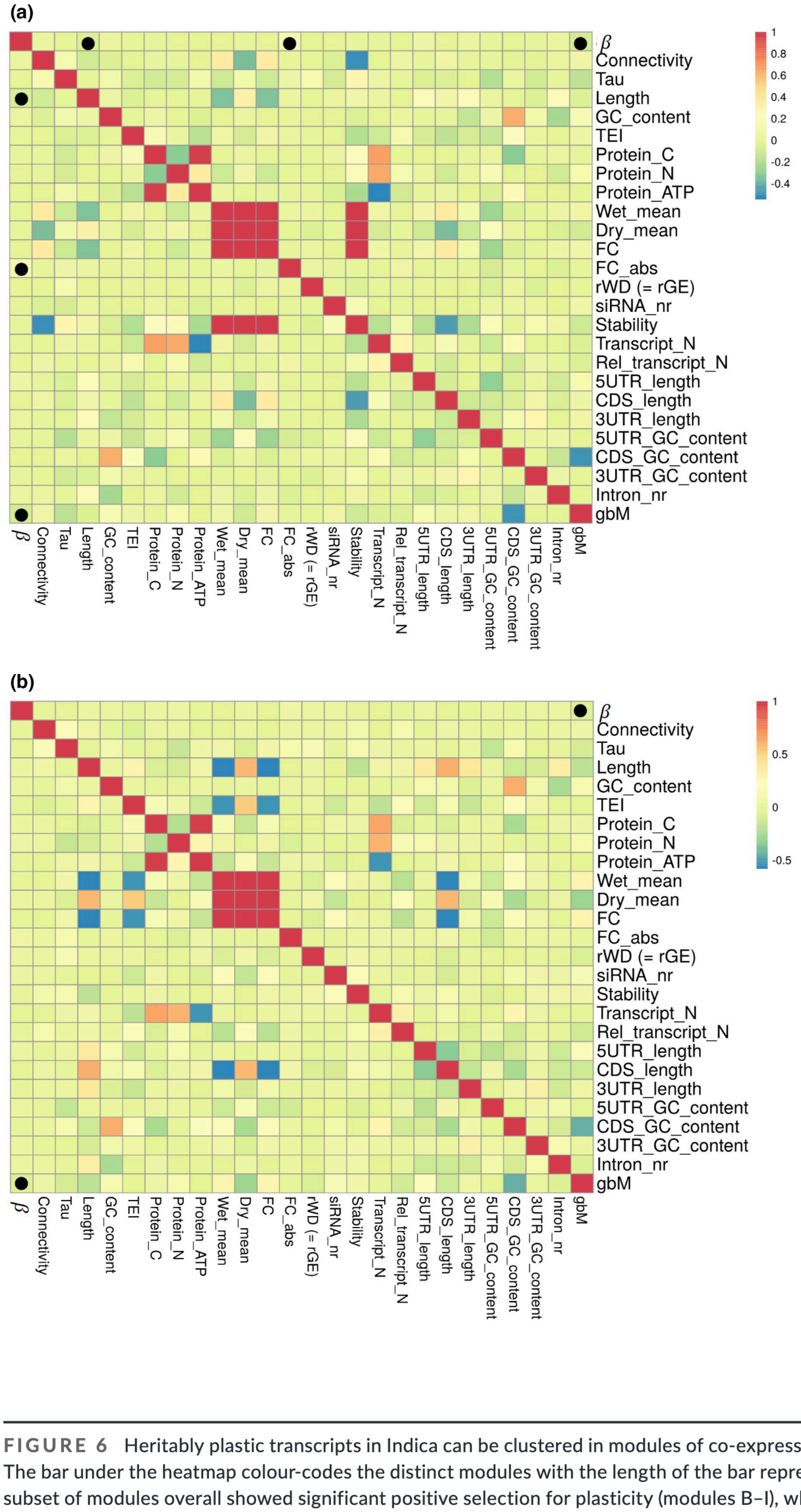

FIGURE 5 Selection on transcript level plasticity showed significant partial correlations with several of 25 architectural, biochemical and network characteristics in Indica (Table S9). The heatmap depicts the level of the partial correlations. Significance is only indicated for partial correlations between β and other factors, and significance was found for gene body methylation (gbM), transcript length (Length), and plasticity level (here defined as the absolute fold change: FC_abs) in GPTs (a) and for gbM in DPTs (b). Statistical information for other partial correlations can be found in Table S9. Dots indicate significant correlations relative to a Bonferroni-corrected $p < 0.0003$.

FIGURE 6 Heritably plastic transcripts in Indica can be clustered in modules of co-expressed transcripts using k-means clustering (a). The bar under the heatmap colour-codes the distinct modules with the length of the bar representing the number genes in each module. A subset of modules overall showed significant positive selection for plasticity (modules B–I), whereas other modules did not (modules A, J–M) (b). Error bars represent 95% confidence intervals around the median; asterisks indicate significance. The modules under selection (B–I) are enriched for photosynthesis-related gene ontology (GO) biological processes as well as processes related to translation and responses to abiotic stress (c). The Y axis represents the negative logarithm of the p value evaluating the significance of enrichment among the modules for each GO term.

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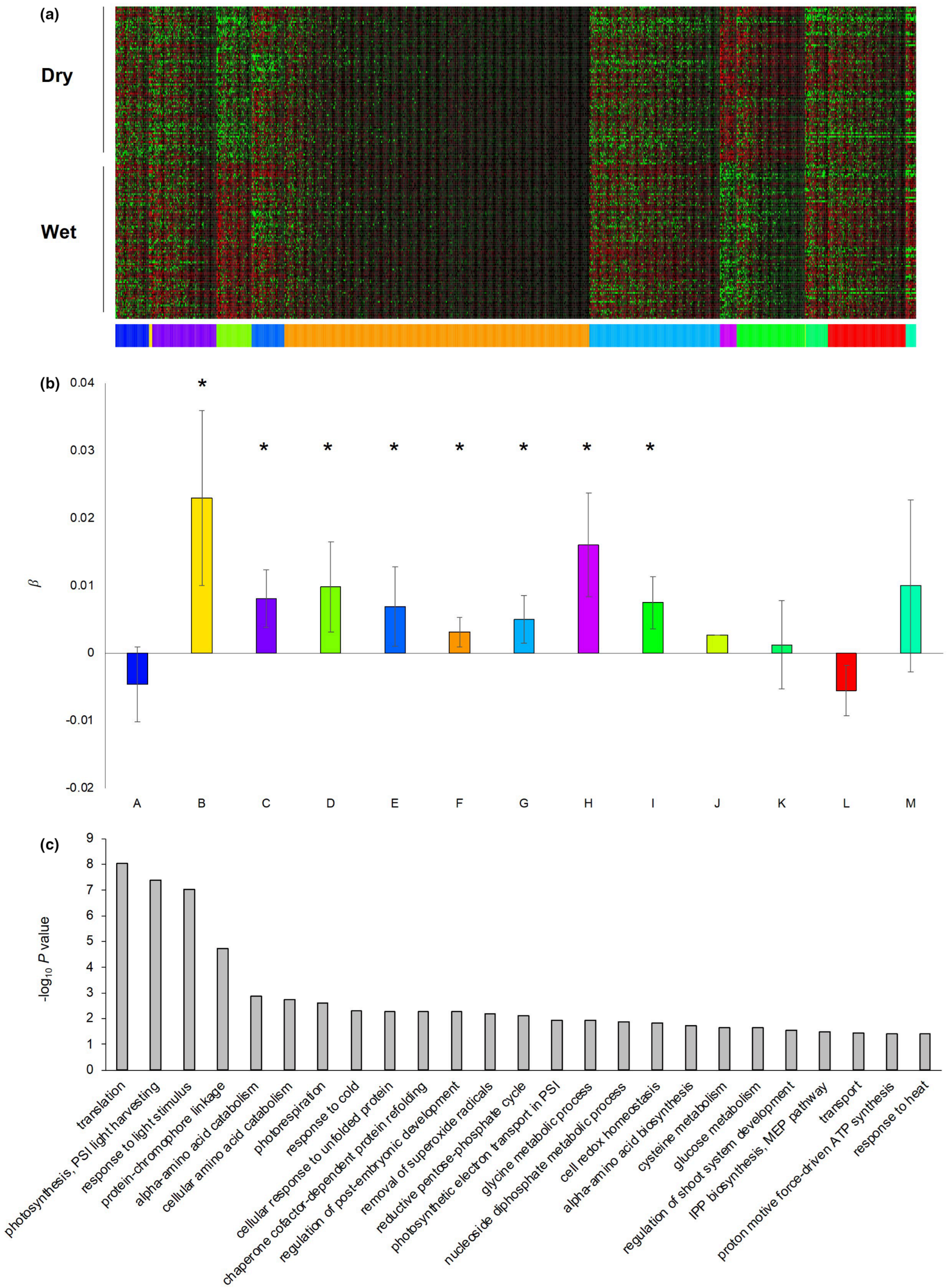

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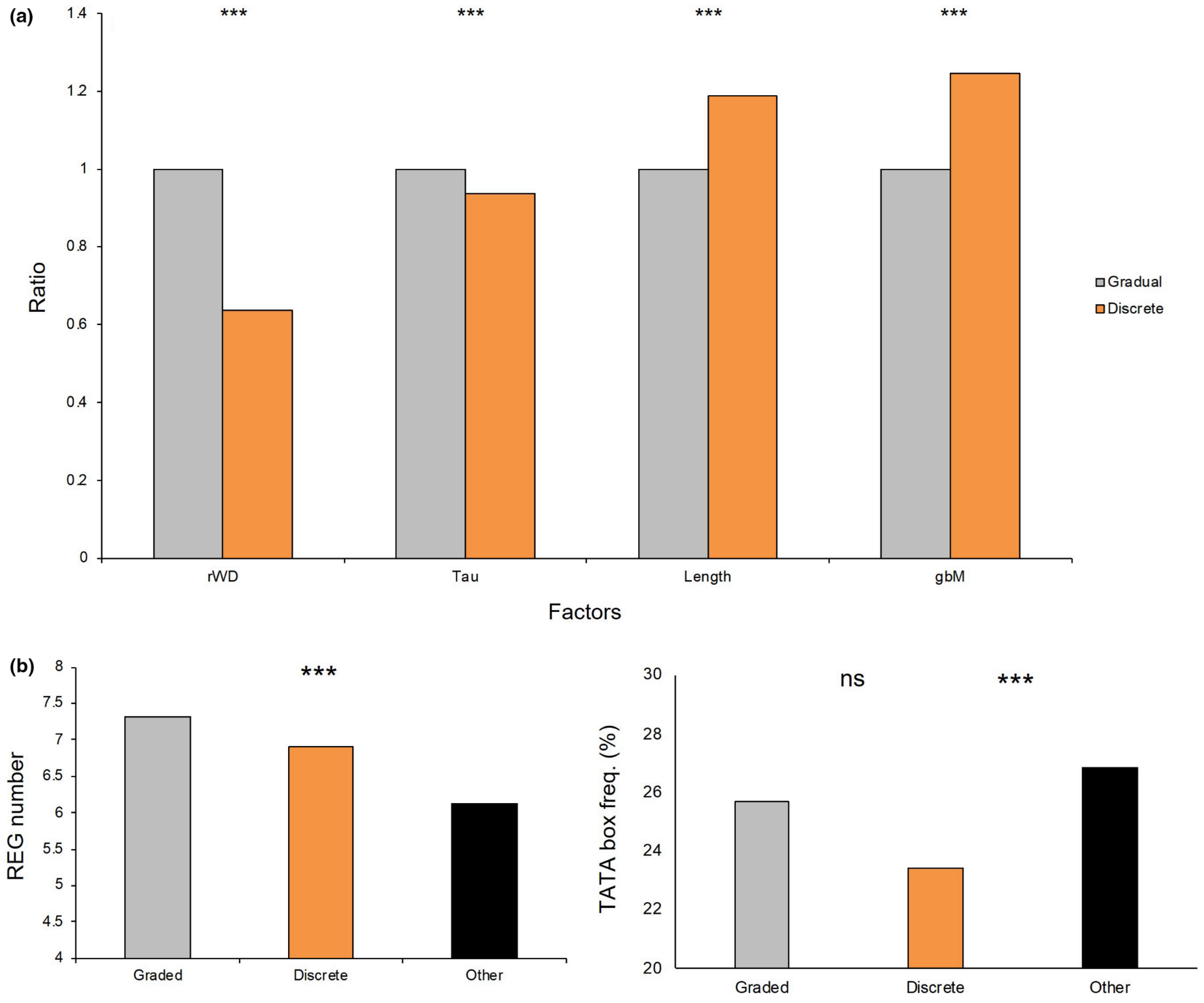

FIGURE 7 Structural and regulatory features of gradually- and discretely-plastic transcripts (GPTs and DPTs respectively) in Indica. Ratios were calculated as the average levels of factors that characterize DPTs relative to average levels of these factors for GPTs (a). Values for the former have been normalized relative to the latter. GPTs and DPTs differed in the number of cis-regulatory promoter elements (REGs) in their genes' promoters (one-way ANOVA; $p < 0.0001$, lower 95% CI for GPTs higher than transcriptome-wide average REG number of 6.65 [6.98, 7.66], which was within 95% CI for DPTs [6.5, 7.31]) (b). GPTs and DPTs also differed significantly in the frequency with which TATA boxes occur in their genes' promoters with respect to the frequency of TATA boxes in the promoters of other leaf-expressed genes (Fisher's exact test; one-tailed $p = 0.127$ for GPTs vs. other transcripts, and one-tailed $p = 0.001$ for DPTs vs. other transcripts respectively). rWD = r_{GE} = cross-environment genetic correlation, Tau = tissue specificity, Length = transcript length, gbM = gene body methylation; ns = not significant, *** indicates $p < 0.001$.

patterns are hypothesized to relax selection on genes, potentially limiting plasticity evolution (Kawecki, 1994; Murren et al., 2015; Roberts & Josephs, 2023). Although the majority of transcripts did not show significant G×E, we did find significant G×E for many transcripts, indicating evolutionary potential. Most drought-induced transcript-level changes occurred in only one or few genotypes, as previously observed in *Arabidopsis thaliana* (Des Marais et al., 2012). We also found that G×E was mostly due to rank-order changes in gene expression levels among genotypes across environments, as has been found for functional traits in *A. thaliana* (Ungerer et al., 2003), *Caenorhabditis elegans* (Gutteling et al., 2007) and *Drosophila melanogaster* (Carreira et al., 2013). Plasticity levels were not significantly correlated with constitutive expression levels, suggesting generally distinct genetic architectures (Auld et al., 2010), as was previously observed for functional traits in maize (Kusmec et al., 2017). Additionally, selection on expression plasticity was neutral or nearly so for most transcripts, resembling the distribution of selection differentials for plasticity of hundreds of functional traits in a wide variety of plant and animal populations (Hendry, 2015; Van Buskirk & Steiner, 2009). These results indicated that although plasticity of expression was neutral in most transcripts, there was selection on plasticity of some transcripts, with many transcripts showing significant G×E, indicating

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evolutionary potential in the plasticity of expression for at least for some genes, which could possibly aid in adaptation.

While plasticity in gene expression appears to be quite common (Hodgins-Davis & Townsend, 2009; Mäkinen et al., 2015), how often gene expression plasticity is adaptive, neutral, or maladaptive is still debated (Bittner et al., 2020; Campbell-Staton et al., 2021; Ghalambor et al., 2015; He et al., 2021). Several previous studies considered genome-wide gene expression plasticity and arrived at opposite conclusions regarding the adaptive value of plasticity when the transcriptome was considered as a unified whole (Bittner et al., 2020; Dayan et al., 2015; Ghalambor et al., 2015; He et al., 2021; Kenkel & Matz, 2016; Mäkinen et al., 2015; Oostra et al., 2018). For example, Bittner et al. (2020) found that plasticity in gene expression facilitates invasion of desert environments by house mice. In contrast, Ghalambor et al. (2015) found that non-adaptive plasticity in Trinidadian guppies potentiates evolution by increasing the strength of directional selection. Similarly, Campbell-Staton et al. (2021) recently highlighted the role of selection against maladaptive heat-induced plasticity during thermal adaptation of *Anolis* lizards to urban heat islands. Another recent study demonstrated that pre-existing plasticity may be a stepping stone for adaptation to stress, but also revealed that the selective forces driving the evolution of gene expression were context-dependent and differed across *Arabidopsis* lineages (He et al., 2021). While these groundbreaking studies did much to deepen our understanding of the adaptive nature of gene expression plasticity, our fine-grained approach enabled us to pinpoint individual transcripts and classes or modules of transcripts for which expression plasticity was associated with changes in plant fitness. For example, one individual transcript in particular showed highly significant positive selection on expression plasticity in Indica. This was the GPT *OS09T0556400-01* from *OsPHT4;5* ($\beta = 0.169$, $p = 3.96 \times 10^{-5}$, Table S9), which codes for an inorganic phosphate transmembrane transporter. *OsPHT4;5* is differentially expressed in response to abiotic stressors (Li et al., 2020), and plastic expression of phosphate transporters contributes to optimization of shoot growth across stressful and non-stressful conditions (Dong et al., 2019). While we cannot directly exclude non-adaptive causal agents (Stoltzfus, 1999), there are several lines of evidence, detailed below, that lead us to suggest that selection can act on gene expression plasticity.

Our genotypic selection analyses showed not only that plasticity was more often selected for rather than against for GPTs, but also that in Indica, expression plasticity was co-gradient with selection on constitutive expression in wet and dry conditions, suggesting that plasticity moves phenotypes closer to their optima, and may be adaptive in Indica rice experiencing variable water availability (Byars et al., 2007). Interestingly, we saw the opposite pattern (a counter-gradient relationship) between expression plasticity and selection on constitutive expression in Japonica. Here, plasticity seemed to be maladaptive, moving phenotypes away from their optima. This difference between varietal groups may be related to the fact that many Indica accessions have been and still are cultivated across agro-ecosystems with strong spatial and temporal variability in water availability (e.g. rainfed lowland and deep-water ecosystems), whereas Japonica accessions tend to be grown in more stably wet irrigated ecosystems, or in upland ecosystems with relatively constant limitations on water availability (Groen et al., 2022). These results suggest that the interplay between gene expression plasticity and the selective outcomes are strongly mediated by the ecological context of accessions, as seen in a recent study comparing the adaptive potential of gene expression plasticity in closely related *Arabidopsis* species (He et al., 2021). A subset of the transcripts under selection for expression plasticity was enriched for regulating known drought-responsive biological processes, in keeping with our previous observation that Indica accessions with broader drought-induced changes in gene expression experience greater fitness in dry conditions (Groen et al., 2020). This result is also consistent with the recent finding that genes with more treatment-specific expression are generally under weaker selection compared to more evenly expressed genes in *Arabidopsis thaliana* (Roberts & Josephs, 2023). Transcripts with beneficial plasticity were further enriched for functionally relevant growth- and photosynthesis-related processes, which are known to be important for fitness (Kromdijk et al., 2016; Wilkins et al., 2016; Yoon et al., 2020). Interestingly, subsets of these genes are regulated by transcription factors such as MYB1R, which evolved changes in expression level following drought in *Brassica rapa* (Hamann et al., 2021), suggesting that these transcription factors may play a key role in drought adaptation in a variety of plant species, and that expression plasticity in these transcripts was adaptive. The evidence for positive selection for gene expression plasticity in certain genes and gene families, which appears to be conserved across several species, indicates that regulatory plasticity is likely to play a crucial role in population establishment and persistence under novel environments. It may also provide avenues to improve crops and engineer organisms to cope with environmental challenges.

While plasticity may be beneficial and under selection for many traits in many contexts, as we found with many GPTs, there are also cases in which plasticity is costly, harmful and selected against, with selection for canalization (reduction in plasticity) instead (Schlichting & Pigliucci, 1998). We found that the majority of DPTs in our analysis showed selection against expression plasticity. This was particularly clear in Japonica, where expression plasticity in DPTs was frequently counter-gradient and thus maladaptive. Stress-induced plasticity is often thought to reveal cryptic genetic variation that increases the genotypic or phenotypic variance otherwise hidden under normal environmental conditions (Ghalambor et al., 2007; Rutherford, 2000). It is likely that the drought stress released more cryptic genetic variation in Japonica compared to Indica, since Japonica accessions are more frequently found in naturally wet irrigated ecosystems. Furthermore, the majority of DPTs were more frequently shut down rather than activated under drought stress conditions, which reduced plant fitness under drought stress. This pattern was likely the result

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of stress-induced dysregulation of gene expression (Kremling et al., 2018). One particular class of genes for which stress-induced repression might result in lower fitness could be the class of genes known as housekeeping genes, such as ones involved in mRNA translation, which are essential for continued growth and development (Deprost et al., 2007). Many architectural features of housekeeping genes seemed to be shared by the DPTs in our analysis. Indeed, housekeeping genes tend to be longer, have wider potential expression across tissues, have fewer TF binding sites and less frequently contain TATA-boxes in their promoters, and display more gene body methylation (gbM) (Aceituno et al., 2008; Wang et al., 2020; Yanai et al., 2005). DPTs showed these exact architectural features, strongly suggesting many may be housekeeping genes, for which stress-induced repression should be selected against. The notion that many DPTs are housekeeping genes is corroborated by enrichment of GO biological processes among them related to intracellular protein transport, mRNA splicing, chromatin remodelling, and other housekeeping processes in both Indica and Japonica rice (Table S16). These results highlight the role of selection against maladaptive regulatory plasticity under stress conditions and rapid climate change adaptation (Campbell-Staton et al., 2021).

Thus, by using genotypic selection analysis on the degree of transcript expression plasticity, we found that while plasticity was neutral for most transcripts, there were some transcripts, especially GPTs, showing selection favouring plasticity, and some transcripts, especially DPTs, showing selection against plasticity. We also used this analysis to investigate potential costs of adaptive plasticity in gene expression. A cost of adaptive plasticity occurs when plasticity is advantageous across environments but reduces fitness within at least one environment (DeWitt et al., 1998; Murren et al., 2015). Such antagonistic effects of plasticity on fitness may constrain the evolution of plasticity, despite its potential advantages for adaptation to heterogeneous environments (Nicotra et al., 2010). Although plasticity costs have been widely theorized, costs have been notoriously difficult to detect (Auld et al., 2010; DeWitt et al., 1998; Relyea, 2002; van Kleunen & Fischer, 2005). Our experimental design allowed us to assess this type of cost of plasticity directly because we had clonal replicates planted in two different environments, allowing us to compare selection on plasticity itself (separate from selection on trait values) within and across environments.

While there were cases in which plasticity of transcript expression was under positive selection across environments, in none of these cases was there also selection against plasticity of that transcript in either environment. Thus, we found no evidence for opposing selection patterns across compared to within environments that could constrain the evolution of plasticity. This finding supports the idea that selection on gene expression plasticity may generally experience relatively limited constraints (Murren et al., 2015), and is in keeping with multiple studies and meta-analyses that found very little evidence for plasticity costs (Arnold et al., 2019; Bergelson & Purrington, 1996; van Kleunen & Fischer, 2005). It is quite striking that out of many transcripts under selection, we found zero evidence for costs of adaptive plasticity. While our study was not designed to determine mechanistically why this was the case, we can surmise why this might be a likely outcome particularly for gene expression plasticity. At the regulatory level, the cost of carrying unexpressed genes or additional regulatory elements is likely negligible in most cases. Supporting this idea, Latta et al. (2012) found no differences in costs associated with amounts of transcription, protein length or ATP production when comparing specialist and generalist responses to environmental salinity differences. This low regulatory cost may help to explain the fact that we did not find a cost of adaptive plasticity for any transcript in this system. While this conclusion should be robust for GPTs, we are more cautious in our interpretation of the results for DPTs, given the fact that we found multicollinearity showing correlations between trait values and plasticity, which is not surprising since expression for these transcripts was on or off in a particular environment. We thus echo the previous call to develop new analytical frameworks to better quantify environment-specific sources of errors (Auld et al., 2010), especially for traits such as expression of DPTs, to improve our understanding of the evolutionary constraints on plasticity at the phenotypic and regulatory level. Additionally, our data are based on leaf samples collected at 50 days after sowing. Results may differ if we were to consider other tissues such as root, shoot or cotyledons, as well as earlier or later developmental stages (Hodgins-Davis & Townsend, 2009). Furthermore, our analytical approach is relatively simplistic, given that we compared selection on plasticity within compared to across environments under experimental conditions where replicated genotypes experienced each environment. However, in natural populations, the true magnitude of such a cost of adaptive plasticity would certainly depend on the frequency with which each environment is encountered, among other factors. As an extreme example, if a population only experienced one environment, then this type of cost, even if it existed under experimental conditions, would not occur at all in the field. While we provide information on the environments experienced by each accession (Table S1), it is beyond the scope of this study to determine how often each accession would encounter each environment in the field. It is difficult to know the frequency of within- and between-growing season shifts in bioclimatic variables that rice varieties have experienced because historical data on this are not always fine-grained enough. In addition, human management of rice plants may have exacerbated gradients in water availability relative to what wild plants would experience when we compare across rice varieties, particularly when contrasting varieties from irrigated versus rainfed agro-ecosystems. Our study populations and experimental set up have more power to detect potential costs of gene expression plasticity than observations on wild plants in natural contexts would have. However, since we do not know the frequencies of shifts in environmental variables that plants in our populations experience, it will be nearly impossible to extrapolate our analyses of how selection may influence the evolution of gene

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expression plasticity on a micro-evolutionary time-scale and make inferences for macro-evolutionary time-scales. Ongoing field studies with a larger panel of accessions from irrigated, rainfed lowland and rainfed upland agro-ecosystems will have more power to address the importance of this issue for the evolution of plasticity. Nevertheless, the fact that we found no overlap in selection for plasticity across environments compared with selection against plasticity within environments points towards a lack of costs of adaptive plasticity at the regulatory level.

4.2 | Factors associated with selection on gene expression plasticity

We examined whether the strength and pattern of selection on gene expression plasticity was linked to factors related to gene architecture, metabolic costs and network features. We found that several factors, including level of plasticity, transcript length and level of gene-body methylation (gbM) appear to influence selection on gene expression plasticity. The effect of gbM is in keeping with a previously observed link between reductions in gbM and adaptive gene expression plasticity in corals (Dixon et al., 2018), and the observation that genes with high gbM levels typically show more consistent expression across cells, and thus low plasticity (Horvath et al., 2019). Such findings have contributed to the view that gbM has homeostatic functions for gene expression (Zilberman, 2017). Interestingly, the factors expression plasticity, transcript length, and gbM together appear to influence longer-term patterns of gene expression and protein evolution in the Poaceae as well (Seymour & Gaut, 2019).

Our network analysis revealed a set of gene modules in Indica with positive average selection on expression plasticity. Transcripts in these modules are enriched in GO biological processes related to photosynthesis, translation and responses to abiotic stress, suggesting selection on expression plasticity of a multitude of genes that participate in these processes. Adjusting photosynthesis and osmotic regulation under fluctuating water availabilities appeared to be particularly beneficial for fitness not only in rice, but also in many other species such as *B. rapa*, where related genes were previously shown to be involved in regulating plastic responses to dehydration (Hamann et al., 2021). Overall, these results suggest selection generally favoured gradual expression plasticity of transcripts associated with biological functions related to photosynthesis, osmotic regulation and stress responses, which all play a critical role in drought-stress responses. In contrast, selection may act more strongly against expression plasticity in housekeeping genes, where dysregulation could have high fitness costs.

To conclude, our study provides a rich, genome-wide view of the factors that shape patterns of selection on gene expression plasticity. Our results offer insights into the interplay between plastic and evolutionary responses to drought at the molecular level. This work can help to pave the way for future functional studies of the underlying mechanisms of the evolution of gene regulation.

AUTHOR CONTRIBUTIONS

S.J.F. and M.D.P. conceived and directed the project; E.H., S.C.G., I.Ć., S.J.F. and M.D.P. designed selection analyses; S.C.G., E.H. and I.Ć. processed fitness, functional trait and gene-expression data; E.H., C.C. and R.K. performed selection analyses; S.C.G., E.H. and T.S.D. performed statistical analyses; S.C.G., E.H., S.J.F. and M.D.P. wrote the manuscript, and all authors edited the manuscript.

ACKNOWLEDGEMENTS

This work was funded in part by grants from the Zegar Family Foundation and the NYU Abu Dhabi Research Institute to M.D.P., the National Science Foundation (DEB-1142784) to S.J.F., and (IOS-1546218 in the Plant Genome Research Program) to S.J.F. and M.D.P., the National Institute of General Medical Sciences of the National Institutes of Health (R35GM151194) and University of California Riverside startup funds to S.C.G., a fellowship from the Swiss National Science Foundation (P2BSP3_168833) to E.H., a fellowship from the National Science Foundation Research Traineeship (NRT) Program Grant DGE-1922642 to T.S.D., and a FCRH Summer Research Grant and Fordham-NYU Research Internship to C.C. We thank New York University High Performance Computing for supplying computational resources. We are grateful to the reviewers for their helpful feedback and to current and former members of the Franks, Groen, and Purugganan laboratories for insightful discussions.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The RNA-seq data are available from SRA BioProject PRJNA588478. Processed RNA expression count data can be found in Zenodo (https://zenodo.org/record/3533431), alongside a sample metadata file with a key to the RNA-seq data in PRJNA588478. Standardized data on gene expression and fecundity have been made available as Supplementary Information and the underlying normalized transcript abundance and raw seed count data has been made available as part of a previously published article (Groen et al., 2020).

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How to cite this article: Hamann, E., Groen, S. C., Dunivant, T. S., Ćalić, I., Cochran, C., Konshok, R., Purugganan, M. D., & Franks, S. J. (2024). Selection on genome-wide gene expression plasticity of rice in wet and dry field environments. *Molecular Ecology*, *00*, e17522. https://doi.org/10.1111/mec.17522

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