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Exploring Stable Low Soil Phosphorous Stress Tolerance in Rice Using Novel Allele Recombination From *Oryza rufipogon*

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ABSTRACT

Advent of changing climatic conditions along with nutrient deficient soils adversely affects the environment for the rice production. Wild introgression lines derived from KMR3 and *Oryza rufipogon* population were evaluated in six environments, including optimum and low phosphorus stress condition. Significant differences among the introgression lines were observed for plant height, tiller number, biomass, grain yield per plant, days to 50% flowering and harvest index across the environments. Based on grain yield observed under optimum phosphorus and limited phosphorus (P stress condition), eight stress tolerance indices were calculated and found STI and GMP are the better indices to discriminate among tolerant and susceptible genotypes, and correlation studies also confirmed the significant association between STI and GMP. Cluster analysis based on stress tolerance indices revealed three different clusters distinguishing genotypes based on their stable performance on yield related traits. AMMI and GGE biplot analysis to identify the stable performance across environments revealed NSR60, NSR101, NSR105, NSR85 and NSR86 as high grain yielders, whereas NSR135, NSR5 and NSR88 as stable performers. WAASBY-based stability analysis on multiple traits (MTSI) showed NSR135, NSR79 and NSR18 with lowest MTSI, indicating their high stability and high mean performance compared with parent KMR3. Further genotyping for low P tolerance gene (*PSTOL1*) and grain yield genes (*Gn1a*, *SPIKE*, *TGW6*, *DEP1* and *OsSPL14*) using allele specific markers showed that the desirable alleles of *SPIKE*, *Gn1a* and *TGW6* were derived from wild parent *O. rufipogon*. Low P tolerance allele *PSTOL1* was absent in recurrent parent KMR3; however, the introgression lines harboured desirable alleles, which were derived from *O. rufipogon*. Further mapping studies will help to identify a significant potential QTLs/gene for low P tolerance from *O. rufipogon*. Wild introgression lines, NSR85, NSR124, NSR80, NSR54, NSR86 and NSR88, were found as the high yielding and nutrient stress tolerant genotypes, which can be used as potential donors in future breeding programmes for low P stress tolerance.

1 | Introduction

Phosphorous (P) is an essential macronutrient required for ATP synthesis, metabolism, plant growth, nucleic acid synthesis (Liu et al. 2015) and plant takes up P in the form of inorganic phosphate. Reduced availability of phosphorous affects plant growth, genetic architecture, physiological activities and reduction in plant height, tiller number, number of panicles, biomass and grain yield per plant (Das et al. 2017). Thus, development of low phosphorus (P) tolerance genotypes

is essential for rice cultivation in P-deficient soils, which are common in many regions of the world. Majority of the rice cultivating soils are acidic in nature with pH range of 5–6. In India, 25-Mha area shows a pH below 5.5 and in 23-Mha area with pH ranging between 5.6 and 6.5 (Mandal 1997). A report by Maji, Obi Reddy, and Sarkar (2012) showed a total of 30.65-Mha land is affected by moderate to strongly acidic conditions. Thus, the focus on developing low P tolerant rice varieties with stable high grain yield, biomass and reduced use of P fertilizers is essential for economic and environmental sustainability of rice cultivation.

Several studies explored the diversity among germplasm and molecular markers for low P tolerance in rice and its wild relatives (Anandan et al. 2022; Roy et al. 2021). *Pup1* is a major QTL identified in *aus* variety Kasalath that confers low P tolerance in rice, and it contains numerous genes that are involved in P signalling and homeostasis. One such gene is *OsAAD*, which encodes an amino acid of dehydrogenase family protein. Yan et al. (2023) reported that the gene *OsAAD* enhances physiological phosphorus use efficiency (PUE) and grain yield by adjusting tillering ability and suggested that it can be a promising gene for increasing the yield in rice under low and recommended phosphorus supply. Similarly, *PSTOL1* gene identified in the *Pup1* QTL region confers low P tolerance via a significant increase in early root growth, root surface area, total root growth and other root modifications, and it enables plants to absorb more nutrients and growth (Wu and Cheng 2014). Studies by Chithrameenal et al. (2018), Swamy et al. (2020) and Anila et al. (2017) demonstrated the marker-assisted introgression of *PSTOL1* gene exhibited an enhanced low P tolerance in rice. Beyond *PSTOL1* several novel loci and haplotypes reported for low P tolerance in rice (Yumnam, Rai, and Tyagi 2017; Pariasca-Tanaka et al. 2014; Neelam et al. 2017). However, further field evaluation, confirmation and validations of those loci were not performed in majority of the studies.

In past, attempts were made to develop low P tolerant lines using available varieties and landraces, and significant improvements were made using marker assisted selection. Another alternate way is the use of wild progenitor species such as *Oryza nivara* and *Oryza rufipogon* that can actively extract and translocate P from soil to plant tissues under low P conditions (Anandan et al. 2022). Wild introgression lines derived from *O. rufipogon* were utilized for grain yield improvement (Ram et al. 2007; Neelam et al. 2016; De Silva et al. 2023), photosynthetic efficiency (Haritha et al. 2017; Hamaoka et al. 2017; Yadavalli et al. 2022), salinity (Quan et al. 2018), drought (Zhang et al. 2006), low P tolerance (Sunanda et al. 2023; Basavaraj et al. 2022) and other biotic, abiotic stress tolerance (Ram et al. 2007; Gu et al. 2012; Atwell, Wang, and Scafaro 2014; Balakrishnan et al. 2022; Balakrishnan, Fukuta, and Neelamraju 2024). Introgression lines derived from wild species were reported to have higher grain yield, P-use efficiency (PUE) and superior performance than cultivars and mega varieties (Balakrishnan, Surapaneni, and Yadavalli 2020; Sunanda et al. 2023; Basavaraj et al. 2022; Magudeeswari et al. 2024). PUE is the ratio of biomass or grain yield to P uptake, and it reflects the ability of plants to acquire and utilize P from the soil (Rose and Wissuwa 2012). Wild introgression lines of rice were observed to have great genetic variations for low P tolerance, root traits and molecular mechanisms that regulate P uptake and transport. Through conventional and molecular breeding, several introgression lines were identified (Basavaraj et al. 2021; Xiang et al. 2015; Basavaraj et al. 2022; Sunanda et al. 2023), and numerous QTLs have been detected from introgression lines for low P stress (Li et al. 2009; Ren et al. 2015).

For a breeding programme, the main objective is to identify and develop stable genotypes for grain yield across varying climatic and soil conditions; hence, G×E interaction study is essential for varietal development (Kempton and Fox 1997; Atlin et al. 2000). AMMI and GGE biplot are excellent tools for visualizing the multi environment studies (Gauch 2006). Additionally, genomic stability of wild introgression lines is to be tested to avoid the reappearance of wild traits in the advanced generations. Most of the agronomic, yield and low P tolerance traits are quantitative in nature. Keeping this in view, introgression lines derived from KMR3/*O. rufipogon* were studied for their stable performance for grain yield and other yield contributing traits by evaluating them across six environments with two different soil P conditions.

2 | Materials and Methods

2.1 | Plant Materials

The experimental material comprised of 135 backcross introgression lines (BILs) derived from a cross between *Oryza sativa cv.* KMR3 (Karnataka Mandya Restorer 3) and wild parent *O. rufipogon*. KMR3 is a stable restorer line used as a parent in KRH2 hybrid development, having bold grain type, and showed a significant reduction for shoot and root dry weight under nutrient stress condition (Das et al. 2017). The *O. rufipogon* accession WR120 was collected from Kerala, India, and maintained at IIRR Hyderabad. The mapping population (NSR) was developed from interspecific hybridization using parents of KRH2 and *O. rufipogon* (IR58025A/*O. rufipogon*//IR58025B///IR58025B//// KMR3) (Marri et al. 2005). IR58025A is a wild abortive cytoplasmic male sterile line of rice, and IR58025B is a maintainer line or isogenic line. In the current study, the advanced mapping population derived from this interspecific multiparental cross was subjected for multienvironment phenotyping and genotyping for yield and low P tolerance.

2.2 | Experimental Design

Backcross introgression lines (BILs) were grown at the Indian Institute of Rice Research, Hyderabad, and the field is situated at 17°19′ north and 78°29′ east and an altitude of 549 m above mean sea level. The materials were evaluated in six environments including four wet season (Kharif) 2014 (E1), 2015 (E2), 2016 (E3) and 2018 (E4) under transplanted optimum soil P conditions and two wet seasons, that is, Kharif 2016 (E5) and 2018 (E6) under low soil phosphorous (P) conditions. Twenty-five days old healthy seedlings were transplanted with 20-cm row spacing and 15-cm plant spacing. Except the fertilizer dose, similar standard packages were practised to maintain proper plant growth and grain yield in all the environments. Recommended dose of NPK (100:60:40 kg/ha) was applied under optimum soil P conditions, and recommended

dose of N:K (100:40 kg/ha) was applied in low soil P conditions. The low P soil plot at IIRR Hyderabad was developed and maintained for more than 35 years without application of phosphorous fertilizer. The available soil phosphorous (Olsen P) is <2 kg/ha, and the genotypes were evaluated in same field in subsequent years. The experimental materials were evaluated in a randomized completed block design (RCBD) with three replications and the same was practised in all six environments.

2.3 | Evaluation of Introgression Lines for low P Tolerance

A 135 BILs evaluated for low P tolerance in Kharif 2016 and 2018 under optimum or recommended P and low P soil condition. The mean yield data recorded under optimum P and low P condition in both seasons were used for identification of tolerant and susceptible lines. Grain yield data recorded under optimum P (Yp) and stress (Ys) condition were used for estimation of stress tolerance indices, namely,

- Stress Tolerance Index—STI = YPYS/(YP)2 (Fernandez 1992),
- Tolerance Index—TOL = YP − YS (Rosielle and Hamblin 1981),
- Stress Susceptibility Index—SSI = (1 − YS/YP)/ (1 − YS/YP) (Fischer and Maurer 1978),
- Yield Stability Index—YSI = YS/YP (Bouslama and Schapaugh 1984),
- Yield Reduction Ratio—YR = 1 − (YS/YP) (Golestani-Araghi and Assad 1998),
- Yield Index—YI = YS/YS (Gavuzzi et al. 1997),
- Per cent yield reduction—PYR = ((YP − YS)/YP) × 100 (Yaseen and Malhi 2009) and
- GMP = (YP × YS) 0.5 (Fernandez 1992),

where YS is the grain yield of genotypes under low soil P condition, YP is the grain yield of genotypes under optimum soil P condition and YS and YP are the mean grain yield of all genotypes under low soil P and optimum soil P conditions, respectively.

2.4 | Evaluation for BILs for Grain Yield Related Traits Under Different Environments

The BILs were evaluated, and data were recorded on five random plants in each replication, and mean data of each replication was used for analysis. The following 10 morphological traits were recorded on five random plants in each replication, that is, days to 50% flowering (DFF), plant height (PH) (cm), number of tillers (NT), number of productive tillers (NPT), panicle weight (PW) (g), biomass (BM) (g), grain yield per plant (YLDP) (g), thousand grain weight (TGW) (g), total dry matter (TDM) (g) and harvest index (HI). Similarly, data were recorded on all the six environments. Based on grain yield, top performing lines, 25 lines were selected and considered for genotyping and stability analysis.

2.5 | Genotyping of top Performing Lines for Low P Tolerance and Grain Yield Related Traits

Genotyping for low P tolerance and grain yield was performed on selected top performing lines. Fresh healthy leaves were collected, and genomic DNA was extracted using CTAB buffer method (Doyle and Doyle 1987). For grain yield traits, a set of nine allele specific markers was used from the previous report by Kim et al. (2016), and for low P tolerance six codominant markers located at the 90-kb InDel region was used (Chin et al. 2011). The PCR reaction mixture consists of 30–50 ng of template DNA (2 μL), 10X PCR assay buffer + $MgCl_2$ (1 μL), 2 mM dNTPs (0.6 μL), 10 pico mole forward and reverse primer (1 μL), 1 unit Taq DNA polymerase (0.1 μL) and nuclease free water. The PCR reaction cycle for yield marker was carried out at 94°C of initial denaturation temperature for 5 min, 94°C of denaturation for 1 min, 55°C of annealing temperature for 1 min and 72°C of extension for 2 min, and 72°C of final extension for 10 min was followed for 35 cycles. For low P tolerance markers, 94°C of initial denaturation temperature for 5 min, 94°C of denaturation for 30 s, 58°C of annealing temperature for 30 s, 72°C of extension for 45 s and 72°C of final extension for 10 min were followed for 35 cycles using BioRad thermal cyclers. Amplified fragments were separated in 2.5% agarose gel, and fragments were visualized in gel documentation unit (VILBER- Bio-Print).

2.6 | Statistical Analysis

The mean phenotypic data obtained from optimum P and low P environments were used for statistical analysis. Descriptive statistics was calculated using STAR ver. 2.0.1. Mean data of grain yield related traits obtained under optimum soil conditions were used for frequency analysis using SR plot (Science and Research plot). Mean data of all the genotypes obtained under optimum soil P condition and low P condition were used for correlation studies using metan package in RStudio. Further boxplot was constructed to check the environmental variations for various grain yield related traits using RStudio package '*ggplot2*'. Comparative study between optimum P (Kharif 2016, 2018) and low P condition (Kharif 2016, 2018) was performed using the mean data obtained. Violin plot was constructed to check the significant difference among lines grown under optimum and low P condition for grain yield related traits. Further stress tolerance indices were calculated using the defined formula, and indices were employed to distinguish the tolerant and susceptible genotypes. Principal component analysis was performed for stress tolerant indices using *prcomp* function, and cluster analysis was performed using *hclust* function in RStudio.

2.7 | Stability Analysis

Top performing 25 BILs tested over six environments were assessed for stability, namely, (1) AMMI model (Gauch and Zobel 1996) and (2) GGE biplot (Yan and Kang 2003). AMMI model uses analysis of variance (ANOVA) for assessing the main effects and principal component analysis (PCA) for the residual effects. The percentage sum of square (% SS) was

calculated by comparing the SS from AMMI ANOVA. The results are presented in AMMI biplot, where the x axis represents the mean yield and y axis represents the PC1 value. The AMMI analytical model was given as per Gauch and Zobel (1996).

$$Y_{ij} = \mu + g_i + e_i + \sum_{n-1}^{n} \lambda_n \alpha_{in} \gamma_{jn} + \theta_{ij}$$

$$\theta_{ij} \sim N\left(0, \sigma^2\right), i = 1, 2, 3, \ldots \ldots, T;\ j = 1, 2, 3, \ldots \ldots, S,$$

where Y_{ij} is the mean yield of i_{th} genotype in j_{th} environment; μ is the general mean; g_i is the i_{th} genotypic effect; e_j is the j_{th} location effect; λ_n is the eigen value of the PCA axis n; α_{in} and γ_{jn} are i_{th} genotype j_{th} environment PCA scores for the PCA axis n; θ_{ij} is the residual; and n is the number of PCA axis in the model.

The GGE biplot displays both genotype (G) and genotype × environment (GE) variation for traits under study (Kang 1993) and based on SREG (sites regression linear model) (Cornelius, Crossa, and Seyedsadr 1996; Crossa, Cornelius, and Yan 2002). The which-won-where biplot displays the various pattern of genotypes and environments in the study. These models were used to interpret and visualize the stability and GEI patterns. In the AMMI model, only the GEI term is absorbed in the multiplicative component, whereas in the GGE model, the main effects of genotypes (G) plus the GEI are absorbed into the multiplicative component.

3 | Results

3.1 | Descriptive Study for Grain Yield Traits Under Optimum P and Low P Soil Conditions

The present study comprised of evaluation of 135 BILs for grain yield and related traits under six environments, including four under optimum soil P conditions and two under low P soil conditions. Under optimum P condition, the DFF ranged between 93.75 (NSR50) and 118.25 (NSR89) days, plant height varied between 73.75 (NSR94) and 153 cm (NSR68) (Table 1). Productive tillers per plant ranged 8.58 (NSR122) to 14.83 (NSR131) and weight of panicle ranged 1.79 g (NSR94) to 3.76 g (NSR121). Grain yield per plant ranged 12.87 g (NSR99) to 27.17 g (NSR78), plant biomass ranged 19.52 g (NSR122) to 46.01 g (NSR85) and thousand grain weight ranged 15.28 g (NSR36) to 26.29 g (NSR132). Harvest index ranged 27.83 g (NSR48) to 49.29 g (NSR122). Similarly, under low soil P conditions, DFF ranged 90.5 days (NSR45) to 137 days (NSR112) and plant height ranged 68 cm (NSR94) to 136.67 cm (NSR124). Number of productive tillers per plant ranged 4.83 (NSR46) to 12.5 (NSR20), panicle weight ranged 1.17 g (NSR104) to 3.13 g (NSR121) and grain yield per plant ranged 4.08 g (NSR9) to 13.9 g (NSR7). Plant biomass ranged 8.91 g (NSR106) to 32.05 g (NSR10), harvest index ranged 15.98 g (NSR5) to 46.65 g (NSR133) and thousand grain weight ranged 13.28 g (NSR37) to 20.28 g (NSR124). Skewness and Kurtosis values showed that the data are normally distributed for all the traits except plant height, days to 50 % flowering under optimum P condition and the data were normally distributed for all traits under low P condition (Figure 1).

3.2 | Correlation Between Grain Yield Traits Under Optimum P and Low P Conditions

Association study was performed on 10 grain yield related traits under optimum P and low P conditions, which are displayed in Figure 2. The results under optimum P condition revealed a significant positive correlation of grain yield with PH ($r = 0.34$), NT ($r = 0.39$), NPT ($r = 0.44$), BM ($r = 0.5$), TDM ($r = 0.81$), TGW ($r = 0.24$), PW ($r = 0.48$) and HI ($r = 0.51$). Biomass exhibited a significant correlation with YLDP ($r = 0.5$), PW ($r = 0.24$), DFF ($r = 0.3$), NT ($r = 0.36$), NPT ($r = 0.39$) and PH ($r = 0.4$). The number of productive tillers showed a positive significant correlation with TDM ($r = 0.47$), BM ($r = 0.39$), PH ($r = 0.17$), YLDP ($r = 0.44$) and NT ($r = 0.97$). Similarly, under low P soil conditions, grain yield exhibited a significant correlation with TDM ($r = 0.58$), HI ($r = 0.46$), BM ($r = 0.3$), PW ($r = 0.25$), NPT ($r = 0.21$) and NT ($r = 0.17$). Biomass showed a positive correlation with TDM ($r = 0.95$), YLDP ($r = 0.3$), PH ($r = 0.48$), NPT ($r = 0.42$), NT ($r = 0.43$) and DFF ($r = 0.31$). The number of productive tillers exhibited a positive significant correlation with NT ($r = 0.98$), TDM ($r = 0.43$), BM ($r = 0.42$) and YLDP ($r = 0.21$).

3.3 | Evaluation of BILs for Stress Tolerance

Mean data of two wet season (kharif 2016 and 2018) obtained under optimum P and low P conditions were used for stress tolerance analysis. Mean data comparison between optimum P and low P condition exhibited a significant variation for all grain yield related traits (Figure 3). Based on the yield data recorded in optimum P and low P condition, eight stress tolerance indices were computed (Table 2), and the result showed highest TOL of 23.08 recorded in NSR100 and lowest of 1.41 recorded on NSR7. YSI ranged between 0.17 (NSR9) and 0.91 (NSR7) and YR ranged between 0.09 (NSR7) and 0.83 (NSR9). Highest STI of 0.93 recorded on NSR88 and lowest of 0.14 recorded on NSR99, SSI recorded highest in NSR9 (1.43) and lowest in NSR7 (0.16), YI observed highest in NSR7 (1.71) and lowest in NSR9 (0.5). Highest percentage of yield reduction (PYR) recorded in NSR9 (83.07) and lowest in NSR7 (9.19), geometric mean productivity (GMP) recorded highest in NSR88 (173.79) and lowest in NSR99 (25.84). Further correlation analysis between grain yield and stress tolerance indices was carried out and represented in Figure 4A. The results showed a positive significant correlation of grain yield under normal condition (YP) with YR ($r = 0.69$), SSI ($r = 0.69$), PYR ($r = 0.69$), GMP ($r = 0.71$), STI ($r = 0.71$), TOL ($r = 0.92$) and negatively correlated with YSI ($r = -0.69$). GMP and STI exhibited a positive significant correlation with YP ($r = 0.71$), TOL ($r = 0.39$), YS ($r = 0.72$) and YI ($r = 0.72$). PYR showed a positive correlation with SSI (r = 1.00), YR (r = 1.00), YP (r = 0.69), TOL (r = 0.9) and negative significantly correlated with YSI (r = −1.00), Ys (r = −0.65), YI (r = −0.65).

Principal component analysis was performed among the stress tolerance indices to discern the contribution of major indices to total variance. The PC1 and PC2 together explained nearly 98.84% to total variation with eigen values greater than 1. Individually PC1 explained 61.91% of variation and PC2 explained 36.93% of variation. Among the indices, SSI, YR, PYR and TOL had contributed highest to PC1 (Figure 4b). Further cluster analysis was carried out to group the individuals into different clusters based on Euclidian distance. The introgression lines were grouped into

TABLE 1 | Descriptive Statistics of wild introgression lines evaluated in six different environments for various agromorphological traits.

Soil condition	ENV	Descriptive	DFF	PH	NT	NPT	PW	YLDP	BM	TGW	TDM	HI
Optimum P	2014 (E1)	Min	91.00	73.00	5.67	5.67	1.53	8.53	14.10	15.49	23.12	26.40
		Max	126.00	160.00	18.00	17.67	4.57	34.85	46.30	25.74	74.55	50.26
		Mean	108.92	126.46	11.94	11.30	3.05	17.97	28.18	20.61	46.15	38.69
		Skewness	0.19	−0.75	0.17	0.28	−0.12	0.62	0.46	−0.71	0.48	0.04
		Kurtosis	0.22	1.79	0.05	−0.08	−0.31	0.48	0.00	3.10	0.02	−0.53
	2015 (E2)	Min	95.00	69.00	6.67	6.67	1.08	9.43	15.92	15.11	32.12	23.89
		Max	125.00	148.00	21.67	21.33	4.31	36.50	58.71	26.13	88.64	51.43
		Mean	114.39	116.31	12.00	11.14	2.60	21.30	34.64	20.04	55.94	38.18
		Skewness	−1.21	−0.56	0.90	1.12	0.06	0.34	0.24	−0.11	0.37	−0.18
		Kurtosis	1.49	0.89	1.84	2.90	−0.64	−0.12	−0.31	1.83	−0.39	0.31
	2016 (E3)	Min	92.00	68.00	7.00	7.00	1.02	5.43	12.57	15.32	18.00	21.54
		Max	125.00	151.67	20.33	19.33	4.40	31.63	50.47	26.50	73.73	49.12
		Mean	114.76	121.72	12.36	11.41	2.38	16.72	32.32	21.49	49.04	33.87
		Skewness	−0.99	−1.11	0.89	0.87	0.48	0.47	0.01	−0.99	−0.12	0.25
		Kurtosis	2.12	5.32	1.29	1.61	1.37	1.02	0.58	2.16	0.76	0.11
	2018 (E4)	Min	88.00	77.33	5.00	5.00	1.80	9.31	14.14	15.19	26.58	21.40
		Max	113.00	159.00	24.33	23.00	4.92	40.96	50.83	26.77	86.51	60.39
		Mean	103.84	131.76	11.06	11.02	3.53	21.98	25.96	21.66	47.94	45.75
		Skewness	−1.24	−0.87	1.18	1.03	−0.07	0.75	0.92	−0.62	0.77	−0.47
		Kurtosis	2.34	4.07	3.53	2.54	−0.35	0.15	0.54	1.67	0.01	0.77
	Mean (optimum P)	**Min**	**93.75**	**73.75**	**8.75**	**8.58**	**1.79**	**12.87**	**19.52**	**15.28**	**34.16**	**27.83**
		Max	**118.25**	**153.00**	**15.33**	**14.83**	**3.76**	**27.17**	**46.01**	**26.29**	**69.84**	**49.29**
		Mean	**110.59**	**124.15**	**11.82**	**11.20**	**2.89**	**19.48**	**30.28**	**20.93**	**49.76**	**39.10**
		Skewness	**−1.34**	**−1.46**	**0.32**	**0.31**	**−0.04**	**−0.03**	**0.23**	**−0.75**	**0.02**	**−0.22**
		Kurtosis	**2.59**	**7.43**	**0.24**	**0.29**	**0.34**	**−0.31**	**1.04**	**3.23**	**0.25**	**0.63**
Low P	2016 (E5)	Min	95.00	69.67	6.33	6.33	0.85	4.10	9.20	14.40	14.57	10.67
		Max	148.00	143.67	17.67	17.67	3.14	14.40	45.87	22.10	54.10	48.29
		Mean	126.70	107.35	11.24	11.00	1.57	7.07	21.53	18.59	28.60	26.02
		Skewness	−0.49	−0.44	0.38	0.42	0.94	1.44	0.42	−0.22	0.50	0.62
		Kurtosis	−0.84	2.43	−0.14	0.03	0.59	2.81	0.42	−0.36	0.51	−0.15
	2018 (E6)	Min	86.00	62.33	3.00	3.00	1.05	2.68	7.87	10.30	11.65	13.38
		Max	126.00	141.33	16.00	14.33	3.53	17.09	34.10	21.80	51.19	48.46
		Mean	107.27	91.62	7.49	7.36	2.24	9.21	13.43	16.90	22.63	40.38
		Skewness	0.54	0.73	0.98	0.78	0.35	0.01	2.23	−0.53	1.14	−1.43
		Kurtosis	−0.25	6.12	2.60	1.37	−0.09	−0.18	8.03	0.06	3.66	2.65
	Mean (low P)	**Min**	**90.50**	**68.00**	**5.17**	**4.83**	**1.17**	**4.08**	**8.91**	**13.28**	**15.25**	**15.98**
		Max	**137.00**	**136.67**	**13.17**	**12.50**	**3.13**	**13.90**	**32.05**	**20.28**	**42.79**	**46.65**
		Mean	**117.01**	**99.47**	**9.40**	**9.21**	**1.91**	**8.10**	**17.55**	**17.72**	**25.66**	**33.03**
		Skewness	**−0.33**	**0.18**	**−0.01**	**−0.19**	**0.63**	**0.38**	**0.49**	**−0.61**	**0.39**	**−0.20**
		Kurtosis	**−0.41**	**3.31**	**0.10**	**−0.02**	**0.18**	**0.93**	**0.39**	**−0.05**	**0.20**	**0.57**

Note: Bold letters indicate mean value.

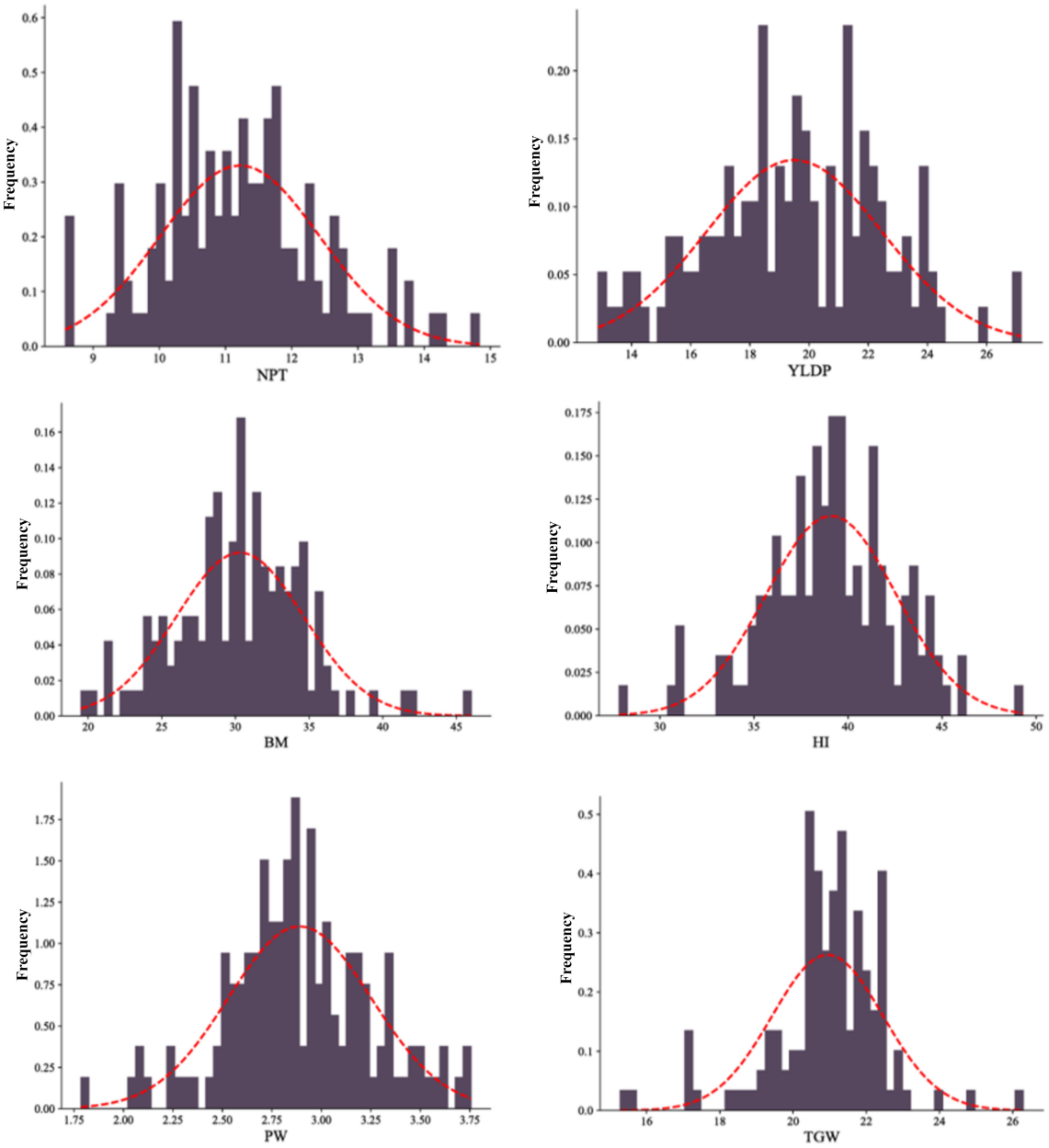


FIGURE 1 | Frequency distribution of wild introgression lines derived from KMR3 x *O. rufipogon* for agromorphological traits.

3 major clusters, cluster 1 consists of 69 lines including parent KMR3 and NSR86 (DRR Dhan 65), cluster 2 consists of 20 lines and cluster 3 consists of 46 lines (Figure 4c).

3.4 | Genotyping BILs for Low P Tolerance and Grain Yield Related Genes

Top performing 25 introgression lines were selected based on grain yield under optimum and low P condition. The selected lines along with parent (KMR3) and checks N22, Swarna, Dular and Kasalath were subjected to genotyping for low P tolerance and grain yield and are represented in Figure 5A. For low P tolerance, six dominant markers, namely, K42, K45, K46-1, K46-2, K48 and K52 located at the 90-kb indel region (Kasalath) on chromosome 12 were used. The marker K42 showed a desirable allele at 918 bp and for K46-2 at 227 bp, and both alleles were present in NSR30, NSR38, NSR105, NSR124, NSR86, Dular and Kasalath. The genotypes NSR30, NSR38, NSR124, NSR86, Dular and Kasalath carry the desirable allele at 276 bp for K45 marker. Desirable allele for K46-1 at 523 bp was present in NSR30, NSR38, NSR96, NSR105, NSR124, NSR86, Dular and Kasalath. Similarly, for K48, the desirable allele at 847 bp was present in NSR30, NSR101, NSR105, NSR124, Swarna, Dular and Kasalath. The genotypes NSR10, NSR30, NSR38, NSR43, NSR79, NSR105, NSR124, NSR86, Dular and Kasalath carried desirable allele at 505 bp for K52 primer. Among the introgression lines screened, NSR30, NSR38, NSR86 and NSR105 were harbouring desirable allele for five primers, and NSR124, Kasalath and Dular were with desirable allele for all six primers (Figure 5B).

Genotyping for grain number gene *Gn1a* was performed by two Indel markers Gn1a-indel1 and Gn1a-indel3, and the desirable

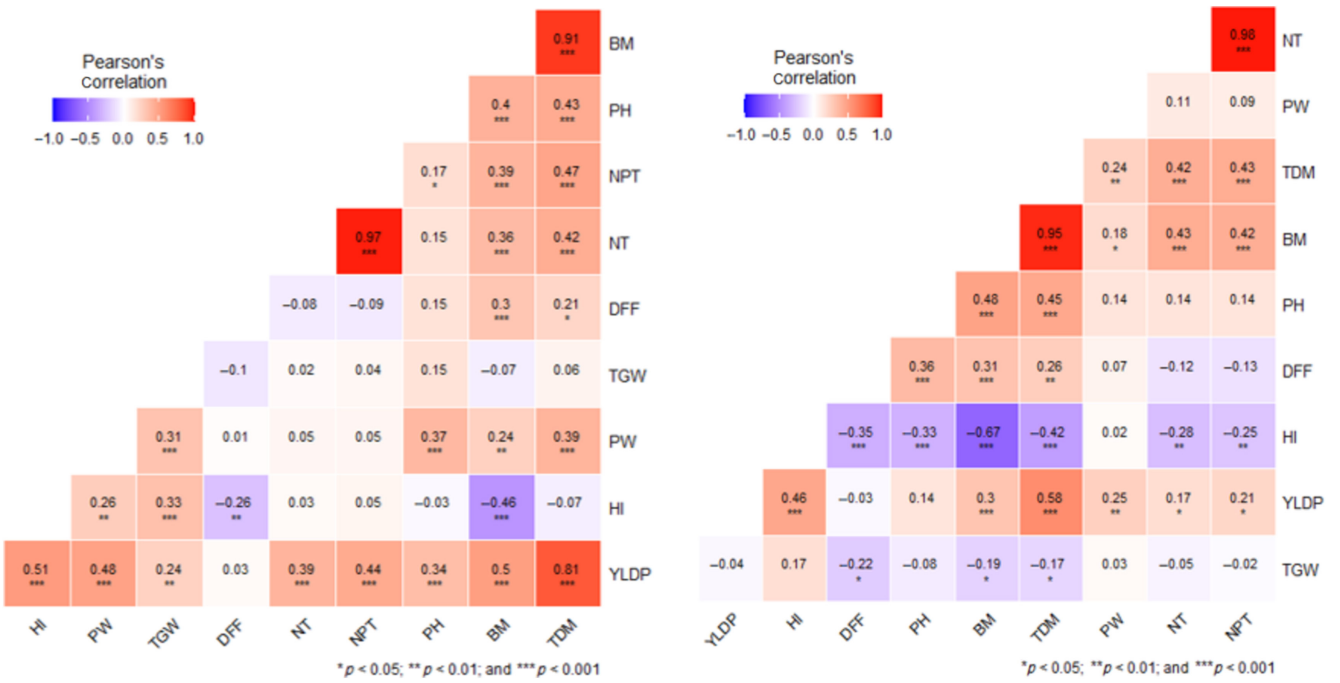


FIGURE 2 | Correlation matrix of introgression lines for various agromorphological traits evaluated under optimum and low soil P conditions. *5% significance; **1% significance and ***0.1% significance.

allele at 99 bp was observed in all introgression lines and checks for Gn1a-indel1. For Gn1a-indel3, the desirable allele was observed in NSR38, Swarna, N22 and Kasalath. For SPIKE-indel3, the desirable allele was observed in NSR1, NSR5, NSR7, NSR83 and N22 at 151 bp. The gene responsible for dense and erect panicle, that is, *DEP1* and panicle architecture gene *OsSPL14*, was entirely absent in all the introgression lines and checks. A gene responsible for thousand grain weight, that is, *TGW6*, was present in NSR38, NSR62, NSR96, NSR101, Swarna and Kasalath. Similarly, gene responsible for grain size, that is, *Gs5* was present in all the introgression lines evaluated. Among the lines, NSR1, NSR5, NSR7, NSR38, NSR96 and NSR101 carried desirable allele for three different grain yield genes, that is, *Gn1a*, *SPIKE* and *TGW6*.

3.5 | Stability of Genotypes Across Various Environments

The introgression lines evaluated under six environments were compared for agromorphological traits showed a significant difference across environments (Figure 6). Based on grain yield per plant data across six environments, 25 introgression lines including parent KMR3 were selected and subjected to stability analysis.

3.5.1 | Grain Yield per Plant

ANOVA indicated a significant difference among all the genotypes for grain yield per plant. Significant mean sum of square (MSS) of genotype revealed that they exhibited a large difference for mean grain yield. Similarly, environment and G×E interaction also exhibited a significant difference. It showed the genotypes behave differently across varied environments/seasons. Among all the environments, genotypic effect accounts for 3.58%, environment effect was 35.62%, interaction effect was 22.89% and 14.49% of residual effect was observed for grain yield per plant. The SS% of AMMI analysis for grain yield per plant, number of productive tillers, thousand grain weight, biomass, panicle weight and harvest index were highly contributed by environment followed by G×E and genotypes.

Discrimination and representativeness graph revealed that the average environmental axes (AEA) are the lines that passes through average environment and biplot origin. The test environment with lesser angle is most representative environment than others. Among the six-environment studied, E2 is the most representative, discriminative environment, and E1 and E4 are the least representative environment. Discrimination of representative environment from others will help in identification of generally adapted genotypes and nonrepresentative environment identifies the specifically adapted genotypes. Genotype evaluation in Figure 7 describes the specific interaction between genotypes and environment. Performance of each genotype in varying environment is visualized in Figure 7. Performance of genotypes in specific environments is presented in Figure 7. In E1, the genotypes NSR78 and NSR41 were performing superior; in E2, NSR135 was performing superior as well as stable genotype. Similarly, in E3, NSR18 and NSR30 were the superior performers; in E4, NSR96 was performing superior. The genotypes NSR7, NSR38, NSR43 and NSR86 were performing superior under low P stress environments (E5 and E6).

3.5.1.1 | **Mean Versus Stability.** The single-arrowed AEA line points out the highest mean grain yield across different environment. Based on this, the genotypes NSR60, NSR101, NSR105

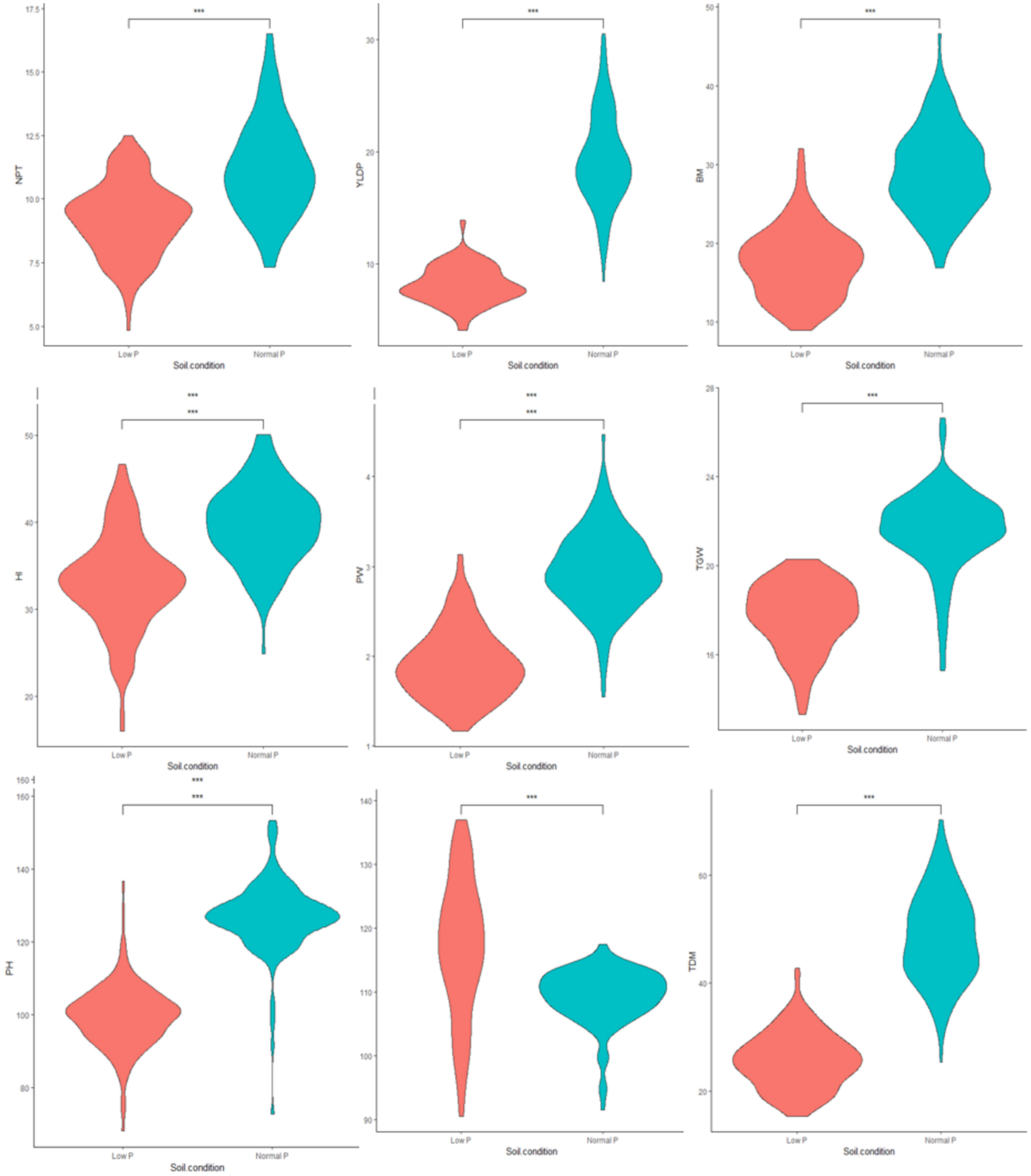


FIGURE 3 | Violin plot describes the performance of introgression lines for agromorphological traits under optimum and low P conditions. Significant differences were represented in * mark. *5% significant difference, **1% significance and ***0.1% significance.

and NSR 85 had highest mean yield, and NSR7 and NSR 43 had lowest mean grain yield across environment. NSR 135 lying exactly on the AEC abscissa showed that it had mean grain yield similar to grand mean. The double arrowed line is AEC ordinate explains its greater variability; that is, less stable and genotype are more uniform, that is, highly stable one across environment. NSR96 expressed greater variability, that is, poor stable line followed by NSR41 and NSR38, whereas NSR135, NSR5 and NSR88 were the more stable ones.

3.5.1.2 | Ranking Genotype Based on Relative to the Ideal Genotype. It revealed the genotype had high mean performance and high ideal stability across environment and are considered as stable or ideal genotypes. An ideal genotype,

TABLE 2 | AMMI analysis of variance (ANOVA) of selected introgression lines for agromorphological traits.

		YLDP	NPT	TGW	BM	PW	HI
Source	**Df**	**Sum of square**					
ENV	5	16,743.74**	1013.50**	1166.96**	22,500.00**	127.30**	15,512.00**
REP (ENV)	12	240.85	29.50	8.46	812.00**	10.12**	507.00
GEN	24	1682.23**	461.00**	1026.78**	6144.00**	25.45**	4255.00**
GEN:ENV	120	10,761.03**	2021.10**	667.62**	16,584.00**	109.33**	13,186.00**
PC1	28	4663.47**	663.40**	339.28**	6762.00**	40.36**	5102.00**
PC2	26	2663.46**	651.50**	154.10**	3732.00**	26.95**	3733.00**
PC3	24	1830.49**	319.90**	79.81**	3046.00**	18.86**	2231.00**
PC4	22	959.17**	251.10**	71.61**	1988.00**	16.24**	1379.00
PC5	20	644.44	135.20	22.82**	1055.00	6.93	740.00
Residuals	288	6812.95	2039.20	310.41	9653.00	66.14	11,995.00
Total	569	47,001.81	7585.40	3847.85	72,276.00	447.68	58,640.00
% SS of genotype		**3.58**	**6.08**	**26.68**	**8.50**	**5.68**	**7.26**
% SS of environment		**35.62**	**13.36**	**30.33**	**31.13**	**28.43**	**26.45**
% SS of G × E		**22.89**	**26.64**	**17.35**	**22.95**	**24.42**	**22.49**

Note: Bold letters indicate mean value.
**Highly significant difference.

that is, genotype falling on the centre of centric circle in a positive direction, also possesses an equal vector length to the longest vector of the genotypes in positive side, that is, high mean performance. Thus, genotypes NSR88, NSR5, NSR135 and NSR 78 are considered as stable. The 'Which Won Where' GGE biplot displays an interaction between genotype and environment and visualizes the winning genotype (Figure 7). The polygon view connecting the genotypes that placed furthest from the origin and dotted lines divide the entire biplot into different sectors and each sector represents a mega environment. For grain yield, six environments have fallen in four mega environments, and for E1 and E2, the winning genotype is NSR41 and NSR78, respectively. Similarly, for E4, the winning genotype is NSR96; for E5 and E6, the winning genotypes are NSR7, NSR86 and NSR43.

3.5.2 | Number of Productive Tillers

ANOVA indicated a significant difference among all genotypes for number of productive tillers. Significant MSS of genotype revealed that they exhibited a large difference for number of productive tillers. Similarly, environment and G × E interaction also exhibited a significant difference. It showed the genotypes behave differently across varied environments/seasons. Among the test environments, E2 is in positive direction and E5 in negative direction. Among the genotypes, NSR41, NSR86 and NSR79 were observed stable genotypes for NPT. The which-won-where graph revealed six environments divided into 4 mega environments and among which in E3, NSR101 found winning genotype; for E1, NSR7, NSR38 and NSR62 were the winning genotypes. Similarly, for E4, NSR135 is the winning genotype.

3.5.3 | Biomass

ANOVA revealed a significant difference among genotypes for biomass. Significant MSS of genotypes revealed that they exhibited a large difference for biomass per plant. Similarly, environment and G × E interaction also exhibited a significant difference. Among the environments, E3 was the least influenced, and E1 and E5 were most influenced. Genotypic view revealed that NSR105 had highest mean values and NSR18, NSR38 and NSR83 had highest biomass; NSR1, NSR7 and NSR79 had lowest biomass per plant. The which-won-where biplot revealed that the six-environment divided into two mega environments, and NSR105 was observed as a winning genotype for E2 and E4. NSR5 and NSR7 were the winning genotypes for E1.

3.5.4 | Harvest Index

ANOVA with significant MSS of genotype revealed that they exhibited a large difference for harvest index. Similarly, environment and G × E interaction also exhibited a significant difference for harvest index. Among the environment, E3 was least influenced, and E6 and E5 were most influenced; among the genotypes, NSR135 was found as a stable genotype. Genotypes NSR30, NSR78, NSR88 and KMR3 observed highest for harvest index, and NSR38, NSR54 and NSR62 were observed lowest for harvest index. The which-won-where graph revealed 2 mega environments, among which NSR38 and NSR86 were the winning genotypes for E5 and E6, and NSR56 and NSR85 were the winning genotypes for E1 and E4.

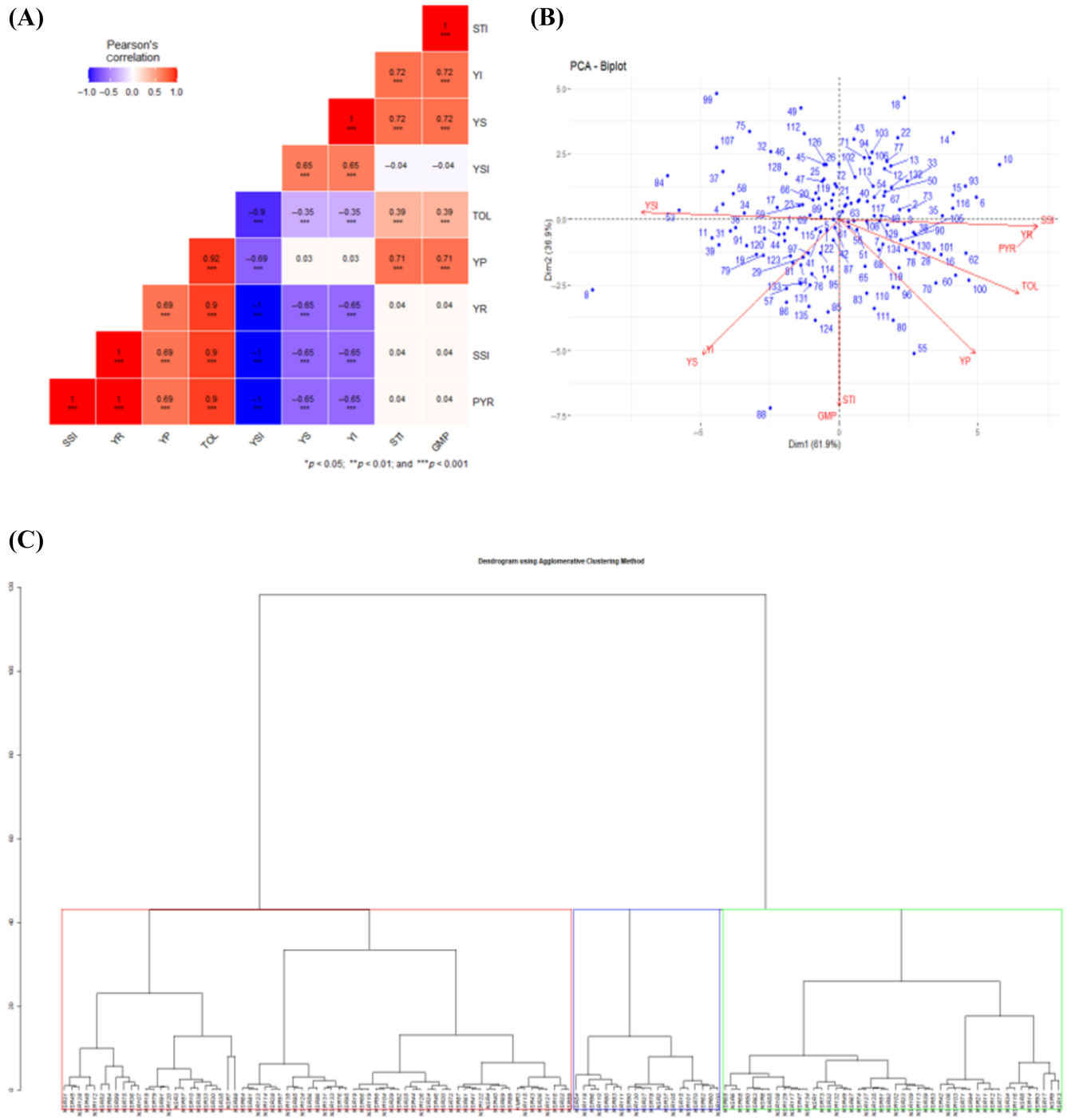


FIGURE 4 | Stress tolerance indices among the introgression lines: (A) Correlation between the stress tolerance indices; (B) principal component analysis explains the association and performance of genotype for each traits; and (C) cluster analysis based on stress tolerance indices.

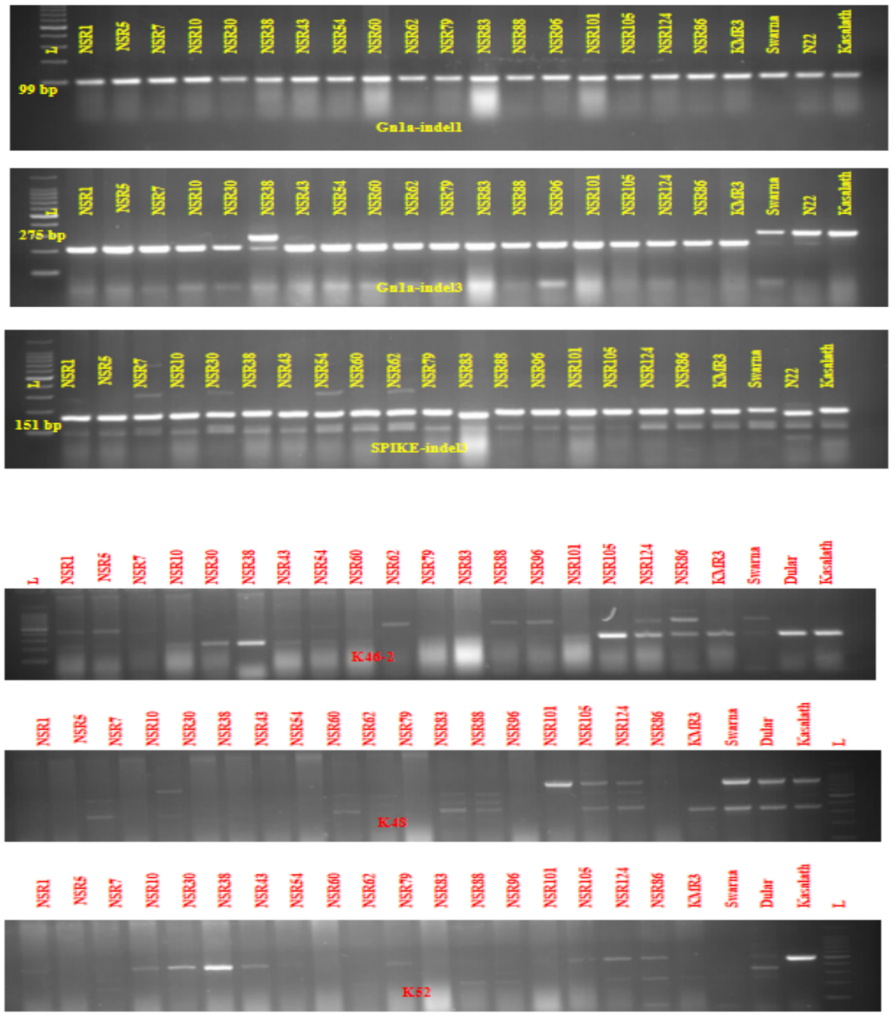
3.5.5 | Panicle Weight

ANOVA with significant MSS of genotype revealed that they exhibited a large difference for panicle weight. Similarly, environment and G×E interaction also exhibited a significant difference for panicle weight. Among the genotypes, NSR1, NSR41, NSR73, NSR79 and NSR96 were the stable genotypes across environment. Genotypes NSR7, NSR10 and NSR56 recorded high panicle weight, and NSR101 and KMR3 recorded low for panicle wight. Among environment, E3 and E4 were most influenced, and E4 and E6 were least influenced. Which-won-where biplot revealed three mega environments, and NSR18 and NSR85 were found as winning genotypes for E2 and E4, respectively. In E1 and E5, NSR5 was observed as a winning genotype, and NSR101 and NSR135 were observed as winning genotypes in E6.

3.5.6 | Thousand Grain Weight

ANOVA with significant MSS of genotype revealed that they exhibited a large difference for thousand grain weight. Similarly, environment and G×E interaction also exhibited a significant

(A)



(B)

	Grain Yield – Allele specific markers							Pup 1 locus specific markers						
Genotypes	Gn1a-indel1	Gn1a-indel3	SPIKE-indel3	DEP-indel1	SPL14-12SNP F/AR	TGW6-1d F/PR	Gs5-03SNP OPF/OPR	K42	K45	K46-1	K46-2	K48	K58	Genotypes
	Gn1a		*SPIKE*	*DEP1*	*OsSPL14*	*TGW6*	*Gs5*	*Pup1*						
NSR1														NSR1
NSR5														NSR5
NSR7														NSR7
NSR10														NSR10
NSR30														NSR30
NSR38														NSR38
NSR43														NSR43
NSR54														NSR54
NSR60														NSR60
NSR62														NSR62
NSR79														NSR79
NSR83														NSR83
NSR88														NSR88
NSR96														NSR96
NSR101														NSR101
NSR105														NSR105
NSR124														NSR124
NSR86 (DRR Dhan 65)														NSR86 (DRR Dhan 65)
KMR3														KMR3
Swarna														Swarna
N22														Dular
Kasalath														Kasalath

FIGURE 5 | (A) Representative gel picture of genotypes screened for grain yield related genes and low P tolerant genes in selected introgression lines; (B) heat map represents the genotyping of selected wild introgression lines for grain yield related genes and low P tolerance genes. Green—desirable allele; blue—undesirable allele.

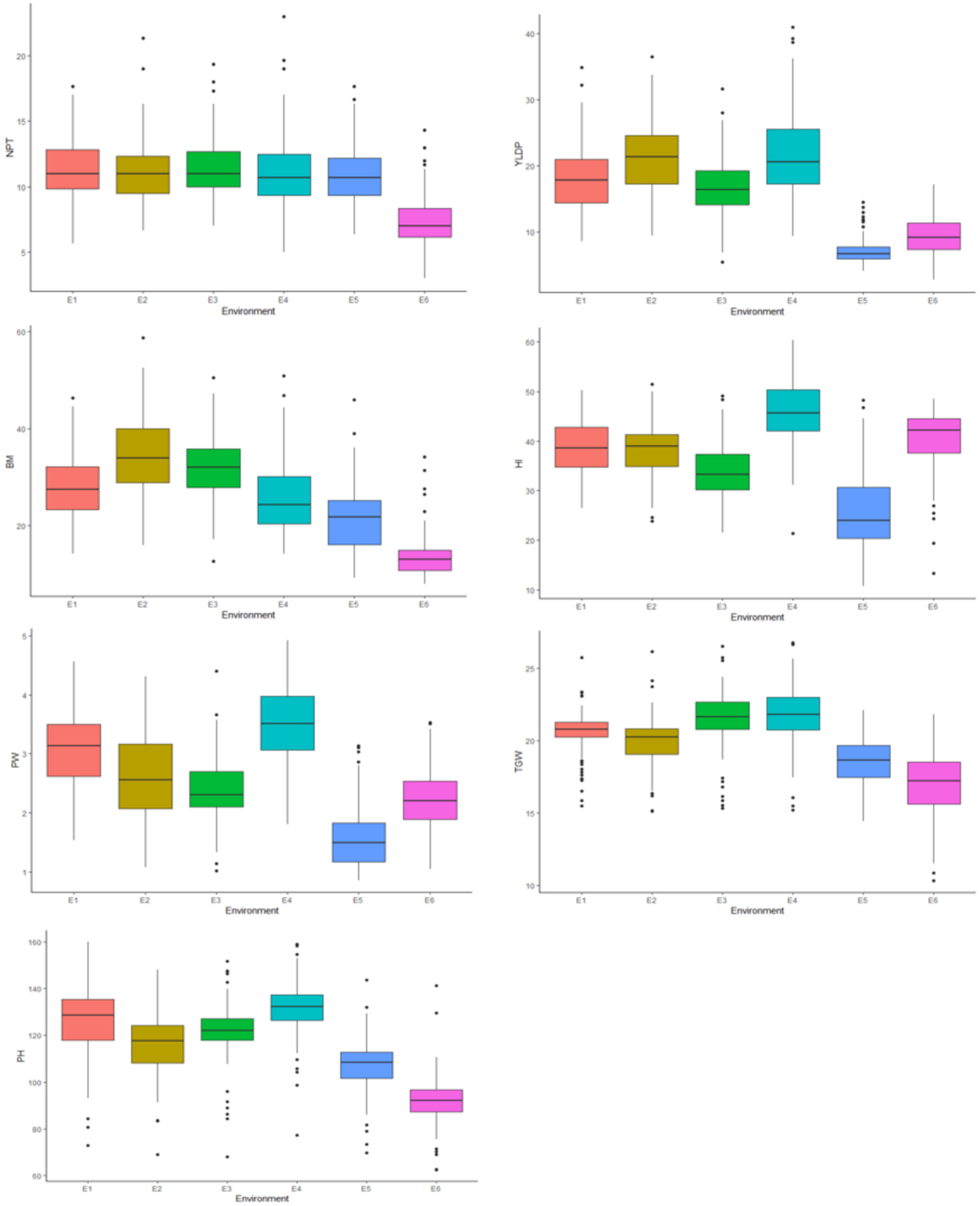


FIGURE 6 | Boxplot represents the significant difference among the six environments for various agromorphological traits.

difference for thousand grain weight. Among the genotypes, NSR56 was observed as a stable genotype, and NSR18, NSR30 and NSR101 showed highest thousand grain weight. Which-won-where biplot revealed three mega environments and NSR5 as a winning genotype in E6; NSR10, NSR43 and NSR124 were found as the winning genotypes in E3. Genotypes NSR30, NSR41 and NSR101 were observed as winning genotypes in E1, E2, E4 and E5.

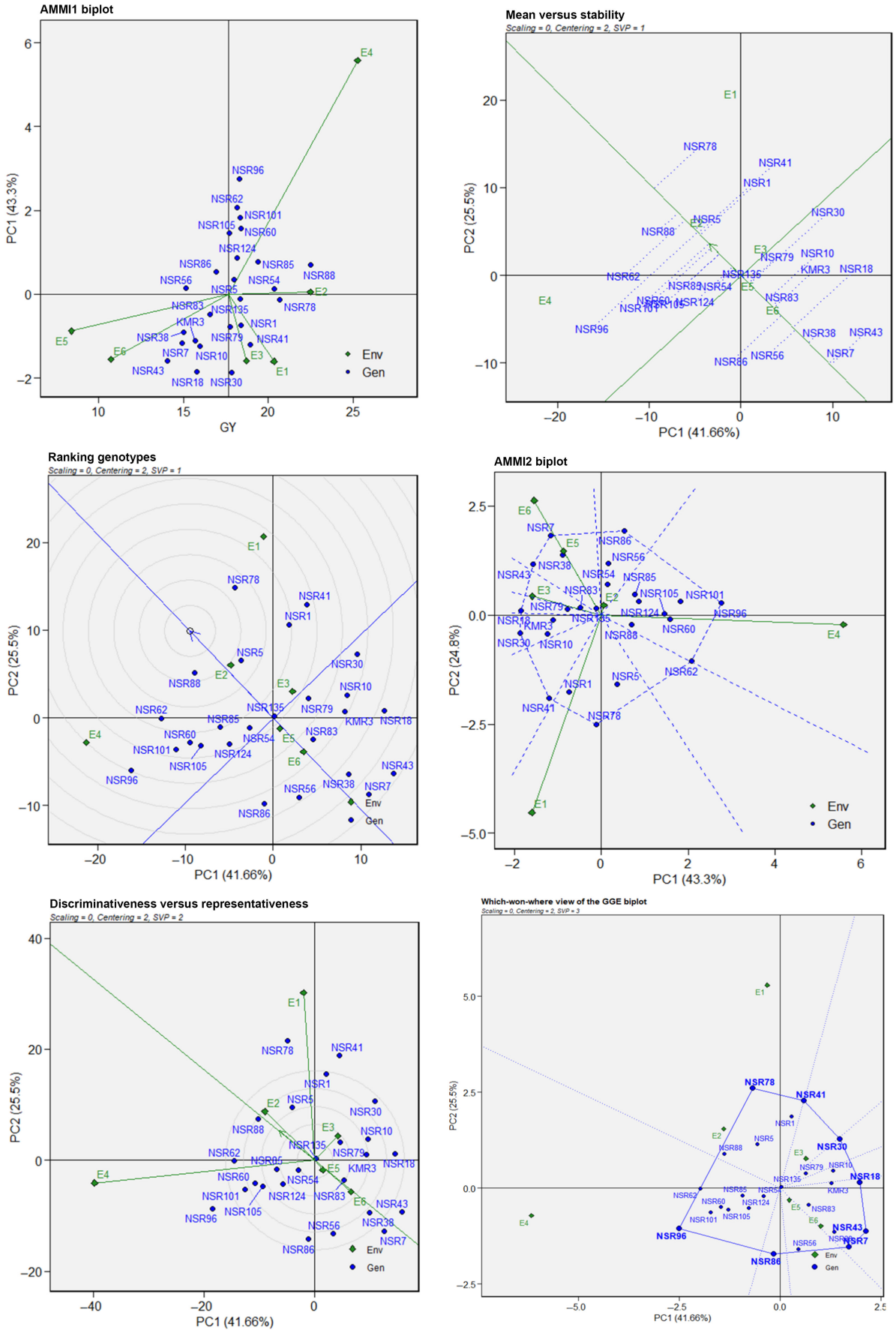


FIGURE 7 | AMMI, GGE and which-won-where biplot view of 25 selected introgression lines for grain yield per plant. E1–E6 indicate the six different environments as described in Section 2.

3.6 | Selection of Superior Genotypes Based on Multitrait Stability Indices

Selection of superior genotypes based on simultaneous selection for combined mean performance and stability of yield related traits (NPT, YLDP, BM, HI, PW, TGW, PH, DFF and TDM) are presented in Figure 8. The genotypes with lowest MTSI values represent high stability and maximum mean values for all the traits selected by considering the selection intensity of 15%. Accordingly, genotypes NSR135, KMR3, NSR79 and NSR18 were the superior genotypes with low MTSI values of 4. Genotypes NSR1, NSR10, NSR62 and NSR43 were recorded with highest MTSI values and represent the poor performance with low stability, as they are located nearer to origin. Heat map

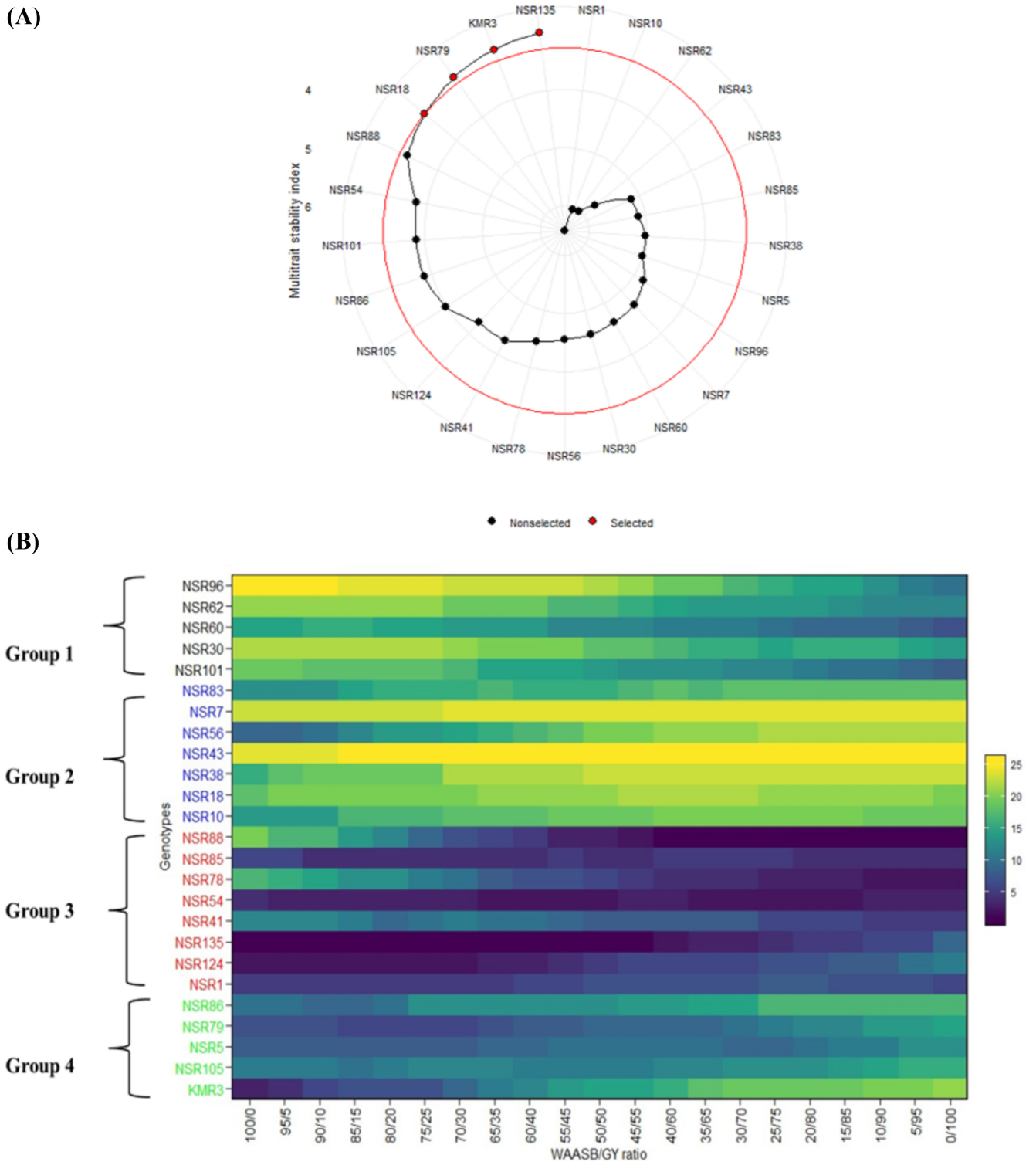


FIGURE 8 | Grain yield, yield related traits performance and stability of selected genotypes across environments. (A) Multitrait stability index (MTSI) of wild introgression lines. Red and black circles indicate selected and nonselected genotypes, respectively. (B) WAASBY indexes showing ranks of the genotypes according to weighting scores of grain yield and stability. List of suitable wild introgression lines in a group.

based on WAASBY ratio, the genotypes were grouped into four groups. Among the four groups, Group 1 consists of genotypes NSR96, NSR62, NSR60, NSR30 and NSR101, and Group 2 consists of genotypes NSR83, NSR7, NSR56, NSR43, NSR38, NSR18 and NSR10. Group 3 consists of genotypes NSR88, NSR85, NSR78, NSR54, NSR41, NSR135, NSR124 and NSR1; Group 4 consists of genotypes NSR86, NSR79, NSR5, NSR105 and KMR3. Among the four groups, the genotypes under Groups 3 and 4 were recommended for varietal development for low P tolerance. Among these genotypes, NSR86 and NSR5 were released as low P tolerant high yielding varieties by Central varietal release committee, as DRRdhan65 (2022) and DRRdhan74 (2024), respectively, for major rice growing states of India after 3 years of evaluation under All India Coordinated Research Project on Rice (AICRPR).

4 | Discussion

Majority of the rice cultivations in Asia and Sub-Saharan African regions are based on low land irrigated conditions leading to an increase in soil acidity (Rakotoson, Tsujimoto, and Nishigaki 2022). Under acidic soil conditions (pH < 6), minerals like phosphorus get fixed to soil particles in organic form, which are not available to plants. Reduced availability of P causes morphological and physiological changes in plants. Wild introgression lines are novel source for low P tolerance as well as high grain yield (Sunanda et al. 2023). Thus, genotyping of introgression lines using low P as well as grain yield related genes was performed using allele specific markers. Besides stress tolerance, identification of stable performance of genotypes for varying soil and climatic condition is essential for successful varietal release.

Under optimum soil P conditions, the descriptive study revealed a significant difference among the introgression lines for all the traits studied. Frequency studies showed a bell-shaped curve revealing that the traits were distributed quantitatively. Further association studies under optimum soil P conditions revealed a significant association of grain yield with all the traits except days to 50% flowering. Biomass showed a significant association with all the traits except thousand-grain weight, and a significant negative correlation was observed with harvest index. Similarly, grain yield exhibited a significant association with all traits except days to 50% flowering, plant height and thousand grain weight. Biomass showed negative significant correlation with thousand grain weight and harvest index under low P stress conditions. Performance of genotypes under optimum and stress conditions revealed a significant reduction for plant height, number of productive tillers, biomass, grain yield, panicle weight and thousand grain weight under P stress condition. The results coincide with the previous reports by Sun et al. (2023), Irfan et al. (2020) and Sunanda et al. (2023).

Introgression lines grown under optimum P and low P conditions were compared for grain yield and its component traits. The significant differences that were observed for all the traits demonstrate the diversity of introgression lines for low P tolerance. Further, variability among the lines was utilized for identifying the tolerant lines using stress tolerant indices. Tolerant index (TOL) defines the difference between grain yield under stress (Ys) and grain yield under optimum condition (Yp) (Rosielle and Hamblin 1981; Basavaraj et al. 2021). Introgression lines NSR7, NSR99, NSR84, NSR52 and NSR107 recorded lowest TOL values represent the highest level of tolerance to low P stress. Selection of genotypes based on low TOL represents high yield potential under P stress condition (Fernandez 1992; Singh et al. 2015) but not high yielding lines. STI is an index used to discriminate high yielding genotypes under stress and optimum condition (Fernandez 1992; Singh et al. 2015). Similarly, GMP is another index for evaluating stress tolerant genotypes. SSI greater than 1 indicates above average stability (Guttieri et al. 2001; Basavaraj et al. 2021). Among these, STI and GMP are better indices compared to TOL and SSI index. Accordingly, NSR85, NSR124, NSR80, NSR54 and NSR88 lines reported highest STI and GMP values. Thus, these lines identified as most stable, productive and tolerant lines among the introgression lines (Ashraf et al. 2015; Basavaraj et al. 2021). Percentage of yield reduction (PYR) recorded lowest in NSR7, NSR84, NSR52, NSR99 and NSR107, and genotypes NSR100, NSR13, NSR93 and NSR9 recorded the highest yield reduction percentage. Thus, the BILs with lowest PYR are more tolerant to low P stress. A high value of YI recorded on NSR38, NSR124, NSR86, NSR88 and NSR7 indicates that these lines were tolerant to stress conditions (Ashraf et al. 2015; Singh et al. 2015). As each index resulted in detection of a different set of tolerant lines; for further confirmation, correlation studies were performed among the indices, and the result showed the highest positive significant correlation was observed between STI with GMP. Similarly, TOL reported a positive significant correlation with SSI, PYR, YR and YP. The index SSI reported a significant association with TOL, YP and YR. A significant positive association of STI with grain yield traits under optimum and P stress conditions revealed the importance of STI as one of the major selection indexes for further advancement for varietal development. Similar results were reported by Basavaraj et al. (2021) and Singh et al. (2015).

Principal component analysis was computed using the stress tolerance indices revealed that PC1 contributed 61.9% and PC2 recorded 36.9%. Eigen values greater than 1 were observed on first two principal components. Among the stress tolerant indices, TOL, PYR, YR, SSI and YP exhibited a positive contribution with PC1, and traits like STI, GMP, YS, YSI and YI exhibited a negative contribution to PC1. Further cluster analysis revealed that the introgression lines were divided into three clusters. Cluster I contained 70 lines including recurrent parent KMR3, Cluster II included 20 introgression lines and cluster III had 46 introgression lines. Mean value comparison of introgression lines across six environments including P stress and nonstress condition revealed a significant difference for number of productive tillers, grain yield per plant, biomass, harvest index, panicle weight, thousand grain weight and plant height. ANOVA revealed that the environments were significantly different for all the traits. Similarly, genotypes, G × E interactions were also found significant for all the traits indicating the importance of multienvironment testing for efficient breeding and adaptability to varying conditions (Atlin et al. 2000; Liang et al. 2015). Thus, the selection based on multienvironment studies improves the efficiency. Varying

degree of G × E interactions were recorded in rice and other cereals crops (Balakrishnan et al. 2016; Pillai et al. 2023; Jadhav et al. 2019; De Silva et al. 2023; Adu et al. 2013). Models like AMMI and GGE biplot analysis were utilized for selecting stable performing introgression lines under multi environment study (Zhang et al. 2019).

AMMI model showed that genotypes NSR60, NSR101, NSR105 and NSR85 reported to have higher grain yield and NSR135, NSR5 and NSR88 were more stable genotypes for grain yield. Similarly, for productive tillers, NSR41, NSR86 and NSR79 reported a stable performance. Introgression lines NSR 105, NSR18, NSR38 and NSR83 reported highest biomass per plant, and NSR30, NSR78, NSR88 and KMR3 recorded highest harvest index per plant. For panicle weight, NSR1, NSR41, NSR73, NSR79 and NSR96 and, for thousand grain weight, NSR56 were identified as the stable genotypes. The genotypes with superior performance but less stable across environment can be stabilized in future by following the limited backcross approach. The genotype that displayed closer to mean environment direction and has less or zero projection on AEC ordinate is considered as ideal genotype, that is, the genotype with high mean yield and high stability. Based on AEA line projection, genotypes NSR60, NSR101, NSR105 and NSR 85 had the highest mean yield, and genotypes NSR135, NSR5 and NSR88 were the stable genotypes. Which-won-where biplot displayed genotypes NSR41, NSR78 and NSR96 as the winning genotypes for grain yield under different environments. Similarly, for biomass, NSR105, NSR5 and NSR7 were the winning genotypes under varying conditions. Further simultaneous selection (WAASBY-based) multitrait stability index (MTSI) effectively identified the ideal genotypes based on combined stability and genotypic performance (Huang et al. 2021; Pour-Aboughadareh et al. 2021). The study showed that NSR135, KMR3, NSR79 and NSR18 recorded the lowest MTSI values, indicating the lines with high stability and high mean performance.

Genotyping of selected introgression lines for allele specific markers *Gn1a-indel1* and *GS5-03SNP-OPF/OPR* revealed that *Gn1a* and *GS5* alleles were present in all the individuals, whereas *DEP1* and *SPL14* were absent in the selected lines. Allele specific markers *Gn1a-indel3*, *SPIKE-indel3* and TGW6-1d F/PR showed segregation among the lines for their respective desirable allele. *Gn1a* increases grain number in rice, and the gene was present in NSR38 and check varieties Swarna, N22, Kasalath (Kim et al. 2016). Similarly, the allele that increases spikelet number per panicle (*SPIKE*) was recorded in NSR1, NSR5, NSR7, NSR83 and N22. The gene that increases thousand grain weight *TGW6* of rice grains was observed in NSR38, NSR62, NSR96, NSR101, Swarna, N22 and Kasalath. The recurrent parent KMR3 carries desirable allele for *Gn1a* and *GS5*. The study concludes that desirable alleles for *SPIKE* and *TGW6* were derived from the wild parent *O. rufipogon*, which enhanced the yield level in introgression lines. It was found that *DEP1* and *OsSPL14* desirable alleles were completely absent in any introgression lines and parent KMR3 indicates the importance of detecting sources and developing novel recombinations in this genetic background. Further, the introgression lines were also screened for low P tolerance using markers in the 90 Kb InDel region of *Pup1* locus (Wissuwa, Yano, and Ae 1998; Wissuwa et al. 2002). Checks Kasalath, Dular and introgression lines NSR30 and NSR124 carried desirable allele for all six markers, whereas NSR38, NSR105 and NSR86 (DRRDhan65) reported to have five desirable alleles for low P tolerance. Interestingly, the parent KMR3 did not amplify the desirable allele for P tolerance, indicating that the desirable alleles were derived from the wild parent *O. rufipogon*. Thus, it revealed that the trait low P tolerance was entirely contributed by wild parent. So further mapping study will help to identify novel QTLs for low P tolerance from wild parent and that can be used in future breeding programmes.

Besides, several significant findings were reported earlier on the introgression lines derived from KMR3/*O. rufipogon*. The introgression lines NSR18, NSR25, NSR36, NSR38 and NSR82 reported for high photosynthetic rate and carboxylation efficiency than recurrent parent KMR3 (Haritha et al. 2017). Similarly, salinity screening studies revealed that NSR105, NSR106, NSR108, NSR114 and Chinsurah Nona2 (IL50-13) were highly tolerant and NSR17 and NSR38 were highly sensitive to salt stress (Ganeshan et al. 2016; Thummala et al. 2022). Grain yield studies revealed NSR18 had highest grain yield per plant and plot yield (Reddy et al. 2012). Based on present and previous studies, NSR38 had recorded highest plant biomass, high yielding and winning genotypes in mega environments, tolerant to low phosphorous stress, and carried desirable alleles for *Gn1a*, *TGW6*, *SPIKE* and *PSTOL1* but sensitive to salt stress. Similarly, NSR18 recorded high biomass, thousand grain weight, grain yield and highly stable performer; NSR105 recorded the highest grain yield and carried desirable allele for low P tolerance.

Till date, three improved introgression lines derived from *O. rufipogon* were released publicly by the Central Variety Release Committee in India. It includes Dhanrasi (C 11-A-41) a low land variety with genes for grain yield, blast resistance, bacterial blight and tungro disease introgressed from *O. rufipogon*. DRRDhan65 (NSR86) (S.O. 4065(E). dt 31st Aug, 2022) a high yielding, low P tolerant variety with multiple stress tolerance and Chinsurah Nona 2 (S.O. 3220 (E) dt 9th July, 2019) a high yielding, salt tolerant variety derived from *O. rufipogon*. Thus, previous reports and released varieties indicate the very high potential of introgression lined derived from interspecific cross of *O. sativa*/*O. rufipogon* for crop improvement as well as identification of novel genes for biotic, abiotic stress tolerance and grain yield genes. To overcome the hybridization barriers and further linkage drag associated with wide crosses, these improved introgression lines can be utilized in future breeding programmes. The introgression lines derived from *O. rufipogon* can serve as a potential donor for novel genes for multiple stress tolerance and yield improvement.

5 | Conclusion

With this collective information, the study concludes the identification of tolerant lines NSR85, NSR124, NSR80, NSR54 and NSR88 for low phosphorus condition (based on STI and GMP) and stable lines for grain yield, namely, NSR135, NSR5 and NSR88. Further, introgression line NSR38 carried desirable alleles for low P tolerance (*PSTOL1*), *TGW6* and *Gn1a*. A high yielding stable line NSR5 harbouring desirable *SPIKE* allele enhanced the number of spikelets per plant. Among the

genotypes, NSR 38, NSR7 and NSR43 were identified as a superior performer under low P stress conditions, and genotypes NSR78, NSR41, NSR18, NSR30 and NSR96 were identified as a superior performer under optimal P conditions. The genotype NSR135 was performed stable in both optimal P and low P environments. To avoid the hybridization problems associated with directly using wild accession, utilization of improved introgression line as a parent in future breeding programme is suggested. By assessing 135 introgression lines, we identified stress environment-specific lines with desirable alleles for tolerance and yield enhancement, which can be effectively employed in breeding programmes.

Author Contributions

P. Magudeeswari: investigation, writing – original draft, software, formal analysis. **Divya Balakrishnan:** conceptualization, methodology, data curation, formal analysis, supervision, funding acquisition, resources, project administration, writing – review and editing, software. **Malathi Surapaneni:** investigation. **A. Krishnam Raju:** investigation. **Yadavalli Venkat Rao:** investigation. **G. Pranay:** investigation. **P. Valarmathi:** investigation. **Vijai Pal Bhadana:** methodology, investigation. **Sarla Neelamraju:** conceptualization, methodology, supervision, funding acquisition, project administration, writing – review and editing. **Raman Meenakshi Sundaram:** visualization, validation, supervision, resources, writing – review and editing.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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