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RESEARCH

Meta-QTL analysis reveals the important genomics regions for biotic stresses, nutritional quality and yield related traits in pearl millet

Shreshth Gupta[1], Sagar Krushnaji Rangari[1], Aakash Sahu[1], Yogesh Dashrath Naik[1], C. Tara Satayavathi[2], Somashekhar Punnuri[3*] and Mahendar Thudi[1,3,4*]

Abstract

Pearl millet (*Cenchrus americanus*) is the sixth most significant cereal crop cultivated on 30 million ha and a staple diet for 90 million poor people across the globe. Besides abiotic stresses several biotic stresses have been limiting production of pearl millet in the semi-arid and arid regions. Although, the Quantitative Trait Loci (QTLs) associated with key diseases like blast, rust and downy mildew resistance and nutritional content has been reported, the use of these QTLs is limited in breeding programs. To identify highly stable consensus genomic regions, we conducted Meta-QTL analysis using 191 QTLs reported in 12 independent studies over the last two decades. As a result, we report 34 Meta-QTLs regions on a consensus genetic map comprising of 692 markers and spanning 2070.7 cM. The confidence interval of Meta-QTLs was reduced by 3.63 folds (0.18–7.49 cM), in contrast to projected QTLs interval of 1.11–60.63 cM. Further, a total of 1198 genes were identified in 34 Meta-QTL regions. Among 34 Meta-QTL regions, Meta-QTL1.1 is found to be region of significant importance as it harbours genes for enhanced biotic stress tolerance, plant growth and development as well as genes related with enhanced seed development. Meta-QTL2.4 has highest number of genes with a significant role in disease resistance which contains basic leucine zipper domain, zinc family, leucine rich repeat regions. Meta-QTL3.1 has ABC transporter like activity coupled with the ATPase activity which has a role in Fe and Zn uptake in leaves and root tissues. These Meta-QTL regions can be used in genomics-assisted breeding for enhancing the blast, rust downy mildew resistance as well as yield and nutritional traits.

Keywords Pearl millet, Downey mildew, Fe and Zn, Meta-QTLs, Confidence interval

*Correspondence:
Somashekhar Punnuri
Punnuris@fvsu.edu
Mahendar Thudi
mahendar.thudi@cgiar.org
[1] Department of Agricultural Biotechnology and Molecular Biology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, India
[2] Indian Council of Agricultural Research (ICAR)-Indian Institute of Millets Research (IIMR), Hyderabad, Telangana, India
[3] College of Agriculture, Family Sciences and Technology, 1005 State University Dr, Fort Valley State University, Fort Valley, GA, USA
[4] Centre for Crop Health, University of Southern Queensland, Toowoomba, Queensland, Australia

Background

In the climate change scenarios attaining sustainable crop production and meeting the global food and nutritional security has been quite challenging. Besides major staple foods, millets are gaining importance and possess the potential to overcome these crises as they are nutritionally rich and are climate resilient. The food security of the world could be stabilized by increasing the production of climate resilient and native crops like pearl millet (Chaturvedi et al. 2022). Recognizing the importance of millets, the United Nations declared year "2023" as the International year of millets. Pearl millet (*Cenchrus americanus* also called as *Pennisetum glaucum,*

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$2n = 2x = 14$) is the second-most important millet after sorghum. It serves as an important alternative crop for feed, food, fodder and relay crop in Brazil, Canada, Mexico, the United States, West Asia and North Africa region and Central Asia (Yadav et al. 2021). Globally it is cultivated on about 30 million ha in more than 30 countries. In India it is cultivated on 6.93 million ha with average production of 8.61 million tons (Satyavathi et al. 2021). Its cultivation may also expand in the maize and sorghum cultivation areas, because of lowering the level of water resources. It is a climate-resilient, nutritionally rich crop and valued for its quality fodder. Its adaptability to harsh climates, including low fertile soil, high pH, high soil Al^{3+} saturation, low phosphorous, low soil moisture, high temperature, high salinity and inadequate rainfall, makes it a versatile and robust choice for agricultural diversification (Gemenet et al. 2015; Kumar et al. 2017; Varshney et al. 2017).

In recent years pearl millet is being preferred as it contains three to five times more nutrition than majority of the cereals and also it is gluten-free and has slow-digesting starch (Serba et al. 2020; Gowda et al. 2022). To escape from drought stress, early maturing cultivars have been developed for drought-prone regions (Yadav et al. 2011) and during the last decade, heat stress tolerance has been reported in the northern and western part of India (Yadav and Rai 2013). But biotic stresses such as downy mildew, rust and blast cause severe damage, which hampers the nutritional content, growth and development of pearl millet (Ambawat et al. 2016; Soriano et al. 2021). Annual grain losses due to downy mildew may be up to 80% (Chelpuri et al. 2019), due to blast disease, it ranges from 10 to 30% (Nayaka et al. 2017) and due to rust it is up to 76% (Ambawat et al. 2016). Compared to other major cereals and legumes, very few efforts were made to identify the promising genomic regions responsible for various biotic and abiotic stresses, agronomic traits and quality related traits in pearl millet. Improvement of agronomic traits like early flowering, bold-seeded and dwarf genotype are the most important factors to increase the seed grain production and to improve the climate-resilience of the pearl millet in harsh conditions (Punnuri et al. 2016; Kumar et al. 2021). Several studies have been conducted to investigate the genetic mechanisms underlying yield and its constituent attributes in pearl millet, leading to the discovery of several quantitative trait loci (QTLs) associated with these traits. For instance, five large effect QTLs for resistance to three different races of the Downy mildew pathogen were reported on linkage group that explained 16.7 to 78.0% phenotypic variance (Chelpuri et al. 2019). In addition, two major blast resistant QTLs on linkage group 4 and 7 of the 863B-P2 line have been discovered by using molecular markers (Singh et al. 2018). The most stable QTL for rust

resistance was identified on linkage group 1, that explained 58% phenotypic variation (Ambawat et al. 2016). Nevertheless, based on 3 years consecutive data (2014–17) from three locations (Delhi, Dharwad, and Jodhpur), 14 QTLs for iron (Fe) and 8 QTLs for zinc (Zn) were identified that explained 2.85 to 19.66% and 2.93 to 25.95% phenotypic variance, respectively (Singhal et al. 2021). Minor QTLs are identified for leaf spot resistance in linkage group 5 and 7, with LOD score above 3 and PVE (Phenotypic Variation Explained) ranging from 4.83 to 5.05% (Punnuri et al. 2016).

Identification of robust consensus genomic regions that harbor QTLs for multiple traits can enhance the transferability of the QTLs for trait improvement though genomics assisted breeding. In recent years, Meta-QTL studies were performed to identify the consensus genomic regions in most of the major crops like wheat (Acuna-Galindo et al. 2015; Soriano et al. 2019), rice (Khahani et al. 2021; Sandhu et al. 2021), maize (Sheoran et al. 2022; Gupta et al. 2023), pulses (Klein et al. 2020; Arriagada et al. 2023) and sorghum (Aquib and Nafis 2022). In the present study we report 34 Meta-QTLs deploying the QTLs studies published over the last two decades. In addition, we also report the key genes in these Meta-QTL regions that can be deployed in pearl millet breeding for developing improved varieties.

Materials and methods
Compilation of QTLs from public domain
An extensive literature search was done to compile QTLs for different traits in pearl millet that were published between 2013 and 2022. These QTLs were systematically grouped into five major trait categories (i) morphological and physiological traits (plant height, canopy structure and water use); (ii) phenological traits (flowering time); (iii) yield and yield related traits (grain size, panicle length, panicle diameter, 1000 grain weight, seed yield per plant, biomass and crop production); (iv) biotic traits (blast, downy mildew and rust) and (v) nutritional traits (Fe and Zn content). The number of QTLs for each trait group ranged from 14 to 93 which is described (Table 1). A total 191 QTLs that contained 136 major QTLs (PVE ≥ 10%) and 55 minor QTLs (PVE < 10%) were selected for the Meta-QTL analysis (Additional file 1). The confidence interval (CI) of the QTLs was calculated using the following equations for recombinant inbred lines (RIL) and F_2 populations (Darvasi and Soller 1997; Guo et al. 2006).

For RIL populations,

$$C.I. = \frac{163}{P \times R^2} \text{ and}$$

For F_2 populations,

Table 1 Summary of QTLs used for Meta-QTL analysis that were reported between 2013 and 2022

Trait	Trait code	Mapping population	Population type	Population size	Number of QTLs	Reference
Morphological and physiological traits						
Plant height	PH	ICMB 841-P3 × 863B-P2	RIL	106	13	Kumar et al. (2017)
		ICMS 8511-S1-17-2-1-1-B-P03 × AIMP 92901-S1-183-2-2-B-08	RIL	317	12	Kumar et al. (2021)
Canopy structure	CS	ICMR1029 × ICMR1004	NIL	162	3	Tharanya et al. (2018)
Water use	WU	ICMR1029 × ICMR1004	NIL	162	4	Tharanya et al. (2018)
Phenological traits						
Days to 50% flowering	FT	ICMB 841-P3 × 863B-P2	RIL	106	6	Kumar et al. (2017)
		Tift 99D2B1 × Tift 454	RIL	186	3	Punnuri et al. (2016)
		ICMS 8511-S1-17-2-1-1-B-P03 × AIMP 92901-S1-183-2-2-B-08	RIL	317	1	Kumar et al. (2021)
Yield and yield related traits						
Grain size	GS	81B × 4025-3-2-B	F_2	188	7	Vengadessan et al. (2013)
Panicle length	PL	ICMB 841-P3 × 863B-P2	RIL	106	11	Kumar et al. (2017)
		ICMS 8511-S1-17-2-1-1-B-P03 × AIMP 92901-S1-183-2-2-B-08	RIL	317	4	Kumar et al. (2021)
		81B × 4025-3-2-B	F_2	188	10	Vengadessan et al. (2013)
Panicle diameter	PD	81B × 4025-3-2-B	F_2	188	5	Vengadessan et al. (2013)
1000 grain weight	TGW	ICMB 841-P3 × 863B-P2	RIL	106	14	Kumar et al. (2017)
		PPMI 683 × PPMI 627	RIL	210	13	Singhal et al. (2022)
		ICMS 8511-S1-17-2-1-1-B-P03 × AIMP 92901-S1-183-2-2-B-08	RIL	317	2	Kumar et al. (2021)
Seed yield per plant	SYPP	PPMI 683 × PPMI 627	RIL	210	14	Singhal et al. (2022)
Biomass	BM	ICMR1029 × ICMR1004	NIL	162	2	Tharanya et al. (2018)
Crop production	CP	ICMR1029 × ICMR1004	NIL	162	11	Tharanya et al. (2018)
Biotic traits						
Blast	BR	J-2537 × ICMB-95444	F_2	36	2	Maganlal et al. (2018)
		Tift 99D2B1 × Tift 454	RIL	186	4	Punnuri et al. (2016)
Downy mildew	DMR	ICMB 89111B-P6 × ICMB 90111B-P6	RIL	187	5	Chelpuri et al. (2019)
Rust	RR	81B-P6 × ICMP 451-P8	RIL	168	3	Ambawat et al. (2016)
Nutritional traits						
Fe content	Fe	ICMB 841-P3 × 863B-P2	RIL	106	3	Kumar et al. (2016)
		ICMS 8511-deriv × AIMP 92901-deriv-08	RIL	317	8	Kumar et al. (2018)
		PPMI 683 × PPMI 627	RIL	210	13	Singhal et al. (2021)
Zn content	Zn	ICMB 841-P3 × 863B-P2	RIL	106	3	Kumar et al. (2016)
		ICMS 8511-deriv × AIMP 92901-deriv-08	RIL	317	7	Kumar et al. (2018)
		PPMI 683 × PPMI 627	RIL	210	8	Singhal et al. (2021)

$$C.I. = \frac{530}{P \times R^2}.$$

where P refers to the size of the population and R^2 refers to the phenotypic variation explained. The absolute, start and end positions of the QTLs were also determined for the QTL projection and the Meta-QTL analysis.

Construction of consensus map

For constructing consensus map, LP merge package in "R studio" was used (Endelman and Plomion 2014). The LP merge package is based on the linear programming which

was used to minimise the error for the markers between linkage groups and the consensus map. The marker name and its position from each linkage map reported in the original QTL studies have been included in the construction of the consensus linkage map. While in case of genetic maps with large number of markers, like Punnuri et al. (2016) we have only used markers flanking the QTLs. The LP merge package in R creates "n" number of models for the consensus map. It creates a weighted as well as unweighted consensus map from the original linkage maps. The best consensus map was selected on the

basis of the least Root Mean Square Error (RMSE) value and the minimum length of the consensus map.

QTL projection and Meta-QTL analysis

The input files containing linkage map and QTL information were independently made for Biomercator v4.2 to perform Meta-QTL analysis. The QTLs were projected using the QTL Projection tool in the Biomercator v4.2 software (Arcade et al. 2004; https://mybiosoftware. com/biomercator-genetic-maps-qtl-integration.html). Meta-QTL analysis was performed using "Veyrieras", a two-step algorithm (Veyrieras et al. 2007) in Biomercator v4.2. In the first step (1/2), the three best parameter values are chosen among the following five parameters i.e. Akaike Information Criterion (AIC), Corrected AIC (AICc), AIC model 3 (AIC3), Bayesian Information Criterion (BIC) and Average Weight of Evidence Criterion (AWE). The best Meta-QTL model, which has the lowest value and highest weight, is selected. In the second step (2/2), the selected model having the number of Meta-QTLs detected is visualized and a file is created by the software that has information on all the detected Meta-QTLs, i.e. the CI, their position and the number of QTLs present within the Meta-QTL.

Detection of candidate genes underlying the Meta-QTL regions

To identify the candidate genes in the Meta-QTL regions, the physical position of the markers flanking Meta-QTL regions was determined using pearl millet genome assembly (Varshney et al. 2017). We retrieved the number of genes from the identified Meta-QTL regions using the information available for pearl millet at Centre of Excellence in Genomes and Systems Biology (https://cegsb.icrisat.org/openaccessdata/). The Gene annotation data of these genes and their predicted function was retrieved using GigaDB database.

Results

The distribution of QTLs from original studies

In order to identify the consensus genomic regions for different traits, a total of 340 QTLs from 17 studies, for 20 different traits were compiled. Of these, we chose 191 QTLs for 16 traits by excluding the QTLs that are reported using RLFP or anonymous markers (Additional file 1). The selected QTLs were from 12 studies containing nine bi-parental mapping populations. The population size of the bi-parental mapping populations varied from 106 to 317 lines (Table 1). Among 191 QTLs, 147 were based on RIL population, 20 were based on near isogenic lines (NIL) population and 24 were based on F_2 population. The number of QTLs per linkage group varied from 16 (on PgLG04) to 51 (on PgLG02) (Additional

file 1). The CI of these 191 QTLs ranged from 1.11 to 60.63 cM (centiMorgan) with an average of 10.86 cM.

QTL projection and construction of a consensus map

The consensus map covers 2070.7 cM distance and contains 692 markers across the seven linkage groups in pearl millet. The generated consensus map consists of SSR (312), SNP (14) and DArT (366) markers (Additional file 2). The length of each linkage group varied from 147.6 cM (PgLG02) to 510.8 cM (PgLG06). The number of markers mapped in each linkage group of consensus map varied from 72 (PgLG03) to 147 (PgLG01). Further, on an average the overall marker density was 0.33 markers/cM while it varied from 0.19 markers/cM (PgLG06) to 0.73 markers/cM (PgLG02) on individual linkage groups (Additional file 3). Over all the QTLs projected on each linkage group varied between 16 (PgLG04) and 51 (PgLG02) (Fig. 1). Minimum of two QTLs (blast resistance) and a maximum of 29 QTLs (1000 grain weight) were projected on the consensus map developed in the present study (Fig. 1).

Identification of Meta-QTLs

Based on the initial QTL projection and the newly developed consensus map, the Meta-QTL regions were determined by using Veyrieras's algorithm. A minimum of two overlapping QTLs associated with minimum two different traits are referred to as the Meta-QTL regions. As a result, of 191 QTLs, 126 QTLs were clustered into 34 Meta-QTLs regions (Fig. 1). Nevertheless, 65 QTLs which were either singlets or that did not have overlapping regions, could not be mapped in any of these 34 identified Meta-QTL regions. Within the seven linkage groups, the highest (7 Meta-QTL) number of Meta-QTLs was identified on PgLG01, PgLG02 and PgLG03, while the least (1 Meta-QTL) number was observed on PgLG07. Further, we identified five Meta-QTLs each on PgLG05 and PgLG06, and two Meta-QTLs on PgLG04. The CI of Meta-QTLs ranged from 0.18 cM (Meta-QTL2.7) to 7.49 cM (Meta-QTL4.2) which is significantly lower than the CI of the projected QTLs (Fig. 2). On an average the CI of Meta-QTLs across all linkage groups is 2.99 cM. So, there is a significant decrease in the CI of Meta-QTLs (2.99 cM) compared to the projected QTLs (10.86 cM). There is a 3.63 fold, or 72.46% decrease in the CI of Meta-QTL compared to the projected QTLs. The markers flanking the Meta–QTL regions are retrieved. Along with the flanking markers, information of position, CI (95%) and number of QTL associated on each Meta-QTL region is summarized (Table 2). The 34 Meta-QTL regions have an average CI of 2.99 cM compared with the average CI of the projected QTLs which were 10.86 cM. A 34.03% reduction in the number of QTLs occurred
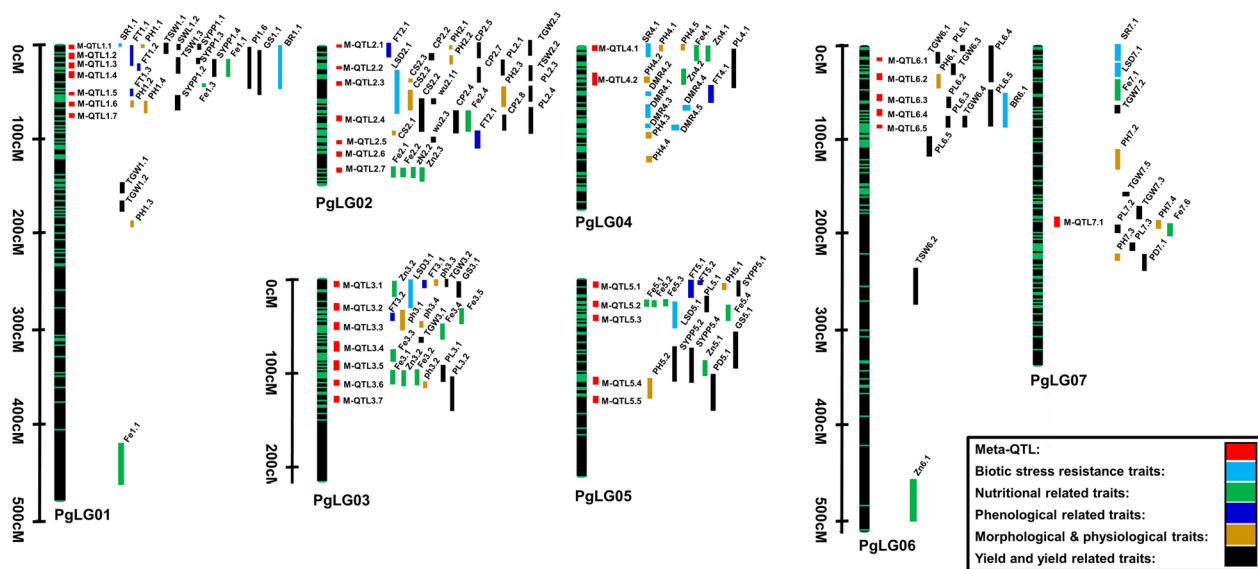
Fig. 1 Consensus genetic map with projected QTLs and Meta-QTLs. Each green line on the graph represents a marker and its corresponding position. The projected QTLs are categorized based on the various traits type, namely biotic stress resistance related traits (Sky-blue), nutritional related traits (Green), phenological related traits (Dark blue), morphological and physiological traits (Saddle brown) and yield and yield related traits (Black). The Meta-QTL region is represented by red colour. The width of each projected QTL and Meta-QTLs corresponds to the confidence interval of them
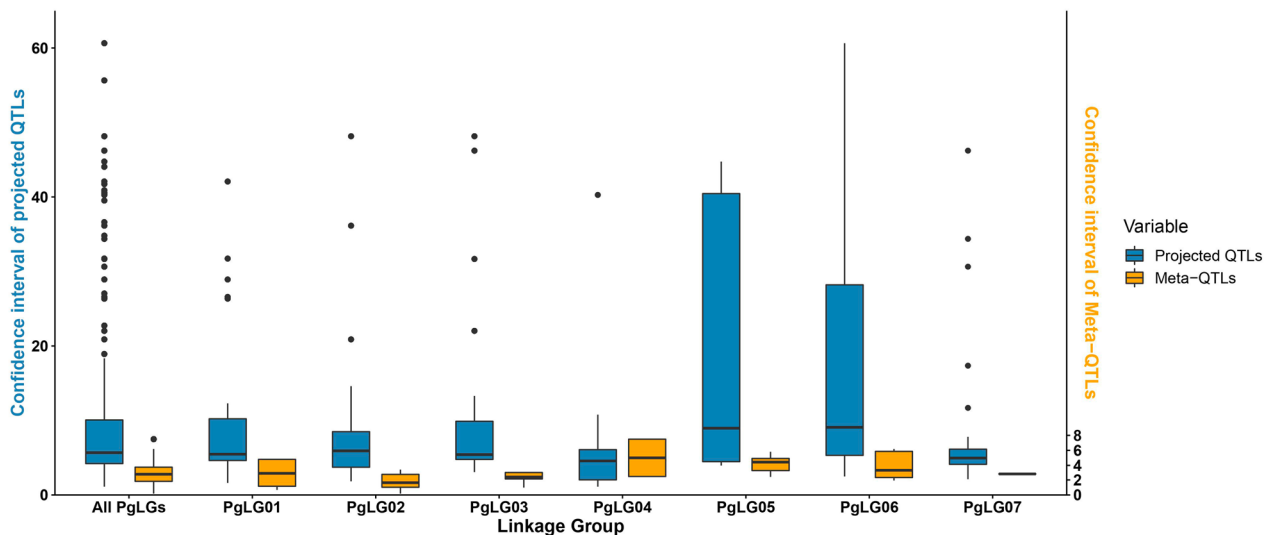

Fig. 2 Graphical representation of projected QTLs and Meta-QTLs. The confidence intervals of projected (represented in steel blue colour) and Meta-QTLs (represented in orange colour) of each linkage group as well as all linkage groups combined (referred to as All PgLGs). The outliers are represented as black dots

compared to the projected QTLs, and these clustered together to form the highly stable Meta-QTL region with the lower confidence interval. These 34 detected Meta-QTLs covers 101.93 cM distance across all the seven linkage groups and are found to be stable and are considered for the further study in the candidate gene identification.

Candidate genes identification from the Meta-QTL region

A total of 1198 genes were identified in 34 Meta-QTL regions on seven linkage groups of pearl millet. The number of genes identified in each Meta-QTL varied from two (Meta-QTL1.4 and Meta-QTL6.2) to 162 (Meta-QTL2.4). However, no genes were found in

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Table 2 Summary of Meta-QTLs and genes identified in this study

Meta-QTL ID	Linkage group	Position			Confidence interval (95%)	Flanking markers	No. of QTL in Meta-QTL	Traits	Number of genes
		Absolute	Start	End					
Meta-QTL1.1	PgLG01	0.17	0	0.85	0.68	IPES0017-S1_3590	8	PH, RR, TSW, PL, SYPP, BR, FT	65
Meta-QTL1.2	PgLG01	16.04	13.69	18.43	4.78	Xipes0017-Xpsms39	4	FT, GS, RR, PL	11
Meta-QTL1.3	PgLG01	22.18	20.315	24.04	3.73	Xpsms39-IPES0042	6	SYPP, GS, RR, PL, TSW	4
Meta-QTL1.4	PgLG01	26.86	24.45	29.26	4.81	Xpsms39-IPES0042	6	Fe, RR, GS, SYPP, TSW, PL	2
Meta-QTL1.5	PgLG01	51.4	50.81	51.98	1.17	Xpsmp2069-PSMP2069	2	Fe, Zn	47
Meta-QTL1.6	PgLG01	61.83	60.38	63.28	2.9	Xismp3017-IPES0098	4	PH, PL, FT	35
Meta-QTL1.7	PgLG01	86.69	85.77	87.6	1.83	pgpb9684-IPES0203	2	PL, SYPP	30
Meta-QTL2.1	PgLG02	0.42	0	1.165	1.49	IPES0123-Xpsmp2201	9	SYPP, CP, TSW, PH	40
Meta-QTL2.2	PgLG02	23.97	23.13	24.81	1.68	Xibmsp31-Xpsmp2231	10	CP, PH, TSW	14
Meta-QTL2.3	PgLG02	58.42	57.595	59.245	1.65	Xpsmp2237-S2_7773	6	WU, BM, BR, CS, PL	23
Meta-QTL2.4	PgLG02	86.81	85.11	88.51	3.4	Xipes027-Xibmcp8	5	BR, WU, CP, PH	162
Meta-QTL2.5	PgLG02	101.86	101.34	102.38	1.04	Xipes0181-pgpb11717	4	PL, WU, CP, BM	13
Meta-QTL2.6	PgLG02	132	130.62	133.38	2.76	pgpb11552-pgpb6832	2	TSW, FT	31
Meta-QTL2.7	PgLG02	139	138.91	139.09	0.18	pgpb6832-IPES0181	6	Fe, Zn	0
Meta-QTL3.1	PgLG03	0.68	0	1.78	2.2	ICMP4014-IPES0161	7	GS, Zn, TSW, PH, BR, FT	20
Meta-QTL3.2	PgLG03	38	36.6	39.4	2.8	Xpsmp2267-PSMP2214	2	PH, FT	104
Meta-QTL3.3	PgLG03	47.98	46.9	49.05	2.15	Xpsmp2227-Xpsmp2214	4	Fe, PH, Zn	8
Meta-QTL3.4	PgLG03	61.91	60.37	63.44	3.07	IPES0233-pgpb9069	2	Fe, TSW	37
Meta-QTL3.5	PgLG03	104.99	103.48	106.49	3.01	IPES0180-Xpsm2222	3	Fe, Zn, PL	108
Meta-QTL3.6	PgLG03	120.71	119.5	121.91	2.41	Xpsms17-IPES0220	6	Fe, Zn, PL, PD	36
Meta-QTL3.7	PgLG03	143.99	143.49	144.48	0.99	pgpb6901-Xpsms31	2	PH, PL	0
Meta-QTL4.1	PgLG04	2.78	1.54	4.02	2.48	Xpsm77-Xipes0225	5	RR, PH, Fe, Zn	27
Meta-QTL4.2	PgLG04	33.81	30.06	37.55	7.49	IPES0208-Xpsmp2084	2	PH, PL	61
Meta-QTL5.1	PgLG05	1.73	0.09	3.36	3.27	S5_1669-Xpsmp2261	5	FT, SYPP, PH	33
Meta-QTL5.2	PgLG05	21.31	20.1	22.52	2.42	IPES0093-Xipes094	6	Fe, SYPP, LSD	31
Meta-QTL5.3	PgLG05	31.44	28.99	33.89	4.9	S5_2145-Xicmp3085	3	BR, PD, Fe	57
Meta-QTL5.4	PgLG05	111.52	109.31	113.72	4.41	Xpsmp2064-Xpsms74	3	Zn, SYPP	4
Meta-QTL5.5	PgLG05	125.83	122.93	128.73	5.8	PSMP2261-IPES0223	4	PH, PD, SYPP	0
Meta-QTL6.1	PgLG06	5.09	4.12	6.06	1.94	ICMP3002-Xipes0167	8	SYPP, TSW, PL, TGW	34
Meta-QTL6.2	PgLG06	27.76	26.59	28.93	2.34	BRP53-IPES0176	2	PH, TSW	2
Meta-QTL6.3	PgLG06	56.28	53.19	59.36	6.17	Xipes035-IPES0085	3	PL, RR	62
Meta-QTL6.4	PgLG06	73.82	70.89	76.74	5.85	BRP90-BRP65	3	PL, RR	50
Meta-QTL6.5	PgLG06	89.62	87.96	91.27	3.31	Xipes0176-Xipes0071	2	TSW, GS	47
Meta-QTL7.1	PgLG07	197.64	196.23	199.05	2.82	Xipes0198-PSMP2263	3	Fe, Zn, TSW	0

Meta-QTL2.7, Meta-QTL3.7, Meta-QTL5.5 and Meta-QTL7.1 (Table 2). Among 1198 genes, 7.93% genes (96) code for proteins with unknown function or domain of unknown function or uncharacterized protein genes (Additional file 3). A total 70, 47, 25, 13, 10 and 6 genes are found across all Meta-QTL regions which are found to be associated with Serine/threonine protein kinase activity, Zinc finger family domain, NB-ARC gene family, F-box cyclin like domain, ABC transporter like activity coupled with the ATPase activity and Basic leucine zipper domain. These genes belong to a wide range of genes family and domains. These functionally annotated

genes with their predicted function can be related to biological pathway which may or may not be associated with traits in the Meta-QTL regions. The candidate genes which code for Serine/threonine protein kinase, NB-ARC, disease resistance protein, pectinesterase, F-box domain, Heat shock proteins, DEAD box domain, NAD(P) binding domain, SANT/Myb domain, No apical meristem protein, Heavy metal associated protein, U–box domain, WD—40 domain, Guanine nucleotide binding protein, Zinc finger protein family, wall associated kinase, etc. has been identified in the 34 Meta-QTL regions (Additional file 4).

Discussion

Globally, pearl millet is the sixth most important cereal crop after rice (*Oryza sativa*), wheat (*Triticum aestivum*), maize (*Zea mays*), barley (*Hordeum vulgare*) and sorghum (*Sorghum bicolor*) (Satyavathi et al. 2021). Being cultivated on 30 million ha and a staple food for 90 million poor people in the arid and semi-arid tropical regions of Asia and Africa, it plays an important role in global food and nutritional security. Although wheat and rice were predominantly grown crops in the post green revolution era, the global food and nutritional demands are not being met alone from the major crops. Although the biological potential of pearl millet is 4–5 tons/ha, it has not been fully realized (Yadav et al. 2021). Pearl millet besides being rich in nutrition, it is a drought hardy crop that withstands higher temperatures and can be grown on marginal soils with minimum inputs (Sehgal et al. 2015; Varshney et al. 2017). Efforts at national and international level in the past two decades provided an understanding of the genomic regions responsible for various traits like blast (Punnuri et al. 2016; Maganlal et al. 2018), downy mildew (Chelpuri et al. 2019), rust (Ambawat et al. 2016), drought tolerance (Sehgal et al. 2015) and nutritional traits have been identified. In addition, HHB67 improved, the first molecular breeding product resistant to downy mildew has been released for commercial cultivation in the case of pearl millet (Hash et al. 2006a, b). Nevertheless, the resistance has been broken down over years and efforts are also made to enhance the resistance. Further, a limited used of the QTLs reported for various traits is seen in pearl millet breeding programs (Gray et al. 2022). It could be due to the non-transferability of these QTLs owing to their background specificity or lack of identification of consensus genomic regions for these traits that enable them to be used in genomics assisted breeding. Identification of consensus genomic regions where major effect QTLs are consistently reported in various studies pinpoint its major role in regulating the particular trait to be further used efficiently in the genomics-assisted breeding program (Sandhu et al. 2021). During

recent years consensus genomic regions were identified using Meta-QTL analysis in several other crops (Acuna-Galindo et al. 2015; Soriano et al. 2019; Klein et al. 2020; Khahani et al. 2021; Sandhu et al. 2021; Aquib and Nafis 2022; Sheoran et al. 2022; Arriagada et al. 2023; Gupta et al. 2023) which enabled the use of the QTLs in enhancing the traits and developing superior cultivars.

To examine the relative positions of QTLs mapped using different molecular marker, a consensus map was constructed by including markers from all the mapping experiments. We used 9 genetic maps to develop a consensus map comprising of 692 markers spanning 2070.7 cM. Earlier genetic maps reported a maximum of 171 markers spanning 898.9 cM (Rajaram et al. 2013). We consider this map as robust consensus map as the marker order was conserved in original maps as well as the marker density increased in individual linkage groups. Similar results were reported in case of wheat where the individual consensus linkage groups are denser than the original linkage map, preserving its original marker order on the individual map (Soriano et al. 2021). Utilizing an integrated consensus map and initial QTL projections, Meta-QTL analysis was conducted. Out of 191 QTLs, 126 QTLs were mapped in the 34 Meta-QTLs region that included 22 morphological and physiological trait QTLs, 8 phenological trait QTLs, 60 yield and yield related trait QTLs, 7 biotic stress and 29 nutritional trait related QTLs (Fig. 1). 34.03% of QTLs could not be mapped in the Meta-QTLs due to lack of flanking or overlapping regions. The CI of the Meta-QTLs is reduced by 3.63 folds or 72.46% decrease compared to reported QTLs in independent studies. Similarly, 5.24 folds reduction of CI was reported in case of rice (Sandhu et al. 2021), 5.2 folds in case of wheat (Soriano et al. 2019) and 46% in case of sorghum (Aquib and Nafis 2022). The higher fold reduction indicates that these Meta-QTL regions are high confidence regions which can be used for introgression and trait improvement. Similarly, such reduction in number of QTLs mapped in Meta-QTL regions and reduction in CI is common, as observed and reported in various crop studies. For instance, in case of wheat, of 368 QTLs only 316 QTLs were mapped in the 84 Meta-QTL regions and CI reduction is 80% (Soriano et al. 2021). While in the case of rice, a significant reduction of 63.2% and 80% in the number and CI of the Zn QTLs, respectively (Joshi et al. 2023).

Key genes in Meta-QTL regions

In the Meta-QTL1.1, 14 *NB–ARC* family associated genes (nucleotide-binding adaptor shared by APAF-1, R proteins and CED-4) play a role in plant growth and development. In previous transcriptomic studies, it has been reported that *NB–ARC* genes play an important

role in the downy mildew resistance response in pearl millet (Kulkarni et al. 2016). In case of rice, *NB–ARC* gene family was reported to be associated with plant panicle development (Pan et al. 2022). Further, the QTLs for panicle development are present in Meta-QTL1.1 which can imply that *NB-ARC* genes can be used for enhancing the panicle development in pearl millet. Further, Meta-QTL1.1 also harbours QTLs for rust and blast resistance where two genes (*Pgl_GLEAN_10018238* and *Pgl_GLEAN_1003685*) that encode for heat shock proteins were also present. Heat shock proteins in plants were reported to act as a chaperone which possess a role in biotic stress tolerance. It plays a crucial role primarily in abiotic stresses such as heat and drought and has also been characterized in pearl millet through transcriptome analysis (Sun et al. 2020). Meta-QTL1.1 also harbours QTLs for flowering time and seed yield related traits. The F–box or cyclin like domain associated genes (*Pgl_GLEAN_10025151* and *Pgl_GLEAN_10018241*) are present in Meta-QTL1.1 were reported to be associated with many biological processes such as pathogen resistance, embroyogenesis, seedling development, floral organogenesis (Xu et al. 2009). These indicate that this Meta-QTL may contribute significantly to biotic stress responses as well as overall development. Two genes (*Pgl_GLEAN_10023785* and *Pgl_GLEAN_10023786*) in Meta-QTL1.6 encode for BURP domain, which was earlier reported to play a role in plant development and is found only in plants (Sun et al. 2019). This Meta-QTL also encompasses genes encoding Leucine-rich repeat (LRR) proteins, which play a crucial role in biotic and abiotic stresses responses. LRRs are recognized as versatile protein recognition domains found in over 14,000 proteins (Matsushima and Miyashita 2012). Hence, this Meta-QTL may play a crucial role in a wide range of adaptability. Identified genes in other Meta-QTL regions, such as those from the multicopper oxidase family (in Meta-QTL6.3), cytochrome P450 family (in Meta-QTL1.1, Meta-QTL2.1, Meta-QTL2.3, Meta-QTL2.4, Meta-QTL3.2, Meta-QTL3.4, Meta-QTL3.6 and Meta-QTL5.2), and ferredoxin reductase family (in Meta-QTL1.5 and Meta-QTL6.3), were previously identified and found to be up-regulated in the transcripts of pearl millet genotypes with high levels of both Fe and Zn (Satyavathi et al. 2022). The cytochrome P450 encoded genes identified in this analysis were previously reported to play a major role in the response to blast disease in pearl millet (Singh et al. 2022a, b). They could be utilized in future breeding programs. The genes *Pgl_GLEAN_10031299* (in Meta-QTL2.4) and *Pgl_GLEAN_10001734* (in Meta-QTL3.2) encode for pathogenic type III effector avirulence factor Avr cleavage site. The Avr proteins enhance host immune response

against pathogen infection (Kim et al. 2009). The genes *Pgl_GLEAN_10037945* and *Pgl_GLEAN_10037946* present in MQTL3.4 and *Pgl_GLEAN_10021100* present in Meta-QTL5.3 are associated with the alcohol dehydrogenase superfamily and are reported to be involved in the development of seeds (Su et al. 2020). The gene *Pgl_GLEAN_10035410* found in Meta-QTL5.2 encodes for U-box protein. In a recent study, U-box proteins were reported to be up-regulated during biotic stress in case of tomato (Sharma and Taganna 2020). The Meta-QTL5.2 harbours QTLs for blast resistance, hence, U-box proteins may be implicated to play a role in blast resistance, which can be further explored in separate study. The MYB gene family represents a significant transcription factor family in plants. These proteins encoded genes were identified within the regions of Meta-QTL1.1, Meta-QTL1.5, Meta-QTL2.2, Meta-QTL2.4, Meta-QTL3.1, Meta-QTL3.4, Meta-QTL3.6, Meta-QTL4.2, Meta-QTL5.3 and Meta-QTL6.3. MYB transcription factors play a crucial role in various plant processes, including responses to biotic and abiotic stresses, development, plant growth, synthesis of secondary metabolites, cell cycle regulation and hormonal signalling (Wang et al. 2021; Chanwala et al. 2023).

The genes that encode ABC transporter like activity coupled with ATPase activity are present in Meta-QTL regions that contained the QTLs for Zn and Fe. In a recent study, using RNA-sequencing data, ABC transporter genes were reported to play a role in Fe and Zn uptake and their transport in leaf and root tissue (Goud et al. 2022). Similarly, 24 genes that encode for LRR are found in Meta-QTLs regions, where the biotic stress resistance QTLs are reported in pearl millet. Six genes (*Pgl_GLEAN_10019857*, *Pgl_GLEAN_10017573*, *Pgl_GLEAN_10020454*, *Pgl_GLEAN_10031316*, *Pgl_GLEAN_10010384* and *Pgl_GLEAN_10027732*) that encode for the basic leucine zipper domain are present in Meta-QTL1.7, Meta-QTL2.4, Meta-QTL3.2, Meta-QTL3.5 were reported to be involved in plant growth and development along with the biotic stress response (Sornaraj et al. 2016). In maize, it has been reported that serine/threonine protein kinase proteins are associated with floret number and ear length, contributing to grain yield. This underscores the significance of this gene in the context of crop improvement (Jia et al. 2020).

Conclusion

We report a consensus genetic map with 34 Meta-QTL regions, where the overall CI is reduced by 3.63 folds or 72.46% compared to the projected QTLs. Among 34 Meta-QTL regions, Meta-QTL1.1 is found to be region of significant importance as it harbours genes for enhanced biotic stress tolerance, plant growth and

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development as well as genes related with enhanced seed development. Meta-QTL2.4 has highest number of genes with a significant role in disease resistance which contains basic leucine zipper domain, zinc family, leucine rich repeat regions. Meta-QTL3.1 has ABC transporter like activity coupled with the ATPase activity which has a role in Fe and Zn uptake in leave and root tissue. These Meta-QTL regions can be used in genomics assisted breeding for enhancing the blast, rust, downy mildew resistance as well as yield and nutritional traits.

Abbreviations

AIC	Akaike Information Criterion
AWE	Average weight of evidence
CI	Confidence interval
cM	Centimorgan
PVE	Phenotypic variance explained
QTL	Quantitative Trait Loci
RIL	Recombinant inbred lines

Supplementary Information

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Additional file 1. Summary of QTLs reported between 2000 to 2022 used for Meta-QTL analysis.

Additional file 2. Maker and their positions of the consensus map developed using 12 genetic maps.

Additional file 3. Genes in the meta—QTL regions and their predicted functions.

Additional file 4. Summary of integrated consensus genetic map of pearl millet developed from 12 studies.

Author contributions
MT and SMP conceived the study; SG, SKR, AS performed the analysis, SG and SKR written the first draft; YDN revised the MS and prepared the Fig. 1; CTS contributed to the resources; MT and SMP wrote the review and edited the MS. All authors read and approved the MS.

Availability of data and materials
All data is provided in the Additional files.

Declarations

Ethics approval and consent to participate
The study does not involve ethical issues.

Consent for publication
All authors read and approved the publication of MS.

Competing interests
Authors declare that there are no financial and competing interests.

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