



Full Length Article

Association Mapping for 100 Seed Weight in Mungbean (*Vigna radiata*) Minicore

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Abstract

Mungbean [*Vigna radiata* (L.) Wilczek] is a protein and carbohydrate rich legume which is mostly grown in Southeast Asia and East Africa. One of the most significant yield contributing traits in mungbean less affected by the environment is 100 seed weight. Mungbean germplasm contains huge amount of variation for this trait. Genome-wide association mapping for 100 seed weight was performed in the World Vegetable Centre's mungbean minicore collection in order to aid in breeding improvement. Around 24,870 single nucleotide polymorphisms (SNPs) were evaluated for the possible association with the seed weight in plants grown in "Pakistan, Bangladesh, Myanmar and Taiwan and potential loci were identified on the chromosomes 1, 4, 6, 8 and 9," which were associated with 100 seed weight. None of the studied markers were stable in all the environments, although the loci on the linkage groups 4 and 8 were found to be important in Pakistan and Bangladesh, while some other loci on the linkage group 8 were found important in plants growing in Myanmar and Taiwan. © 2022 Friends Science Publishers

Keywords: GWAM; GWAS; Linkage; Mungbean; 100 Seed weight

Introduction

The mungbean, commonly referred to as green gram, is a significant legume crop grown in East Africa and Southeast Asia. It is currently grown on roughly 7.3 million hectares around the world, yielding 5.3 million tonnes on an average of 0.73 tonnes per hectare. India, China and Myanmar are the main producers of mungbean. Mungbean yields on average in Pakistan are equivalent to those worldwide, although the country produces lesser overall yields of mungbean due to the limited area that is planted. Mungbean is a well-liked legume crop due to its short vegetative phase, resilience to drought stress, capacity to fix atmospheric nitrogen, and high nutrient content. On a daily food requirement basis, 100 grams of mungbean grains provide 23.9 g of protein, 62.6 g of carbs, 16.3 g of dietary fibre and 1.15 g of fat, along with around 4.8 mg of Vitamin C, 132 mg of calcium, 189 mg of magnesium, 367 mg of phosphorus, 6.74 mg of iron and 2.68 mg of zinc (USDA 2019). Other plant parts besides grain are utilised as manure and feed. Mungbean is suitable for a wide variety of cropping patterns and combinations because to its brief cropping period (Nair and Schreinemachers 2020).

A 100 seed weight in the range of 5 to 6 grams and higher is frequently favoured because large seeded mungbean commands premium pricing on the market. This is due to the fact that seed weight influences both the quantity and the

quality of the crop (Dahiya *et al.* 2015). The high seed weight in Pakistan indicates that the environmental conditions in Pakistan are most favourable for the mungbean production. This is why, the average production of mungbean in Pakistan is not only comparable to the average production of the world but it has also surpassed the average mungbean yield globally. According to a recent report of the World Vegetable Center, the current average mungbean yield is 0.73 t/ha (World Vegetable Center 2022), while according to the report of the Economic Survey of Pakistan, the average mungbean yield during the year 2020–2021 was 0.88 t/ha (GOP 2021). This indicates that the average yield of mungbean in Pakistan is more than the global average.

Different studies show that seed weight in mungbean is not much influenced by the environment. For instance, Yimram *et al.* (2009) reported a genotypic variance of 1.64, and environmental variance of 0.14, along with phenotypic coefficient of variation 29.40 and genotypic coefficient of variation 28.21, which indicated that most of the variation in seed weight is due to genotype as compared to environmental influence. Similarly, Akbar *et al.* (2018) suggested breeding following by selection at early generations for improving the 100 seed weight in mungbean. In addition to this, breeding for greater grain size would be made easier with the knowledge of the genetic loci across different environment that contribute towards this feature. The screening as well as

the selection of the parents for the production of high yielding and disease resistant mungbean genotypes can be aided by molecular markers connected to several features (Collard and Mackill 2008). In bi-parental populations, loci on different chromosomes related to mungbean seed weight have previously been mapped (Isemura *et al.* 2012; Alam *et al.* 2014). Genome-wide association studies (GWAS) or genome wide association mapping (GWAM) are complementary genetic methods that screen more alleles than bi-parental populations in order to identify the changes in the phenotype in the germplasm panels. Due to its low environmental influence, high broad sense heritability and the genetic advance, 100 seed weight is one of the ideal variables for association mapping investigations (Yimram *et al.* 2009).

In order to find out the potential single nucleotide polymorphisms (SNPs) that are directly linked to the 100 seed weight in mungbean, a genome-wide marker set was tested in the current study in a mungbean minicore collection at four different locations, namely Pakistan, Bangladesh, Myanmar, and Taiwan. The objectives of the present study were to a) estimate number of sub-populations which the studied mungbean minicore belongs, b) determine the distribution of average seed weight across different locations and c) screen out various SNPs potentially associated with 100 SW in mungbean minicore.

Materials and Methods

Around 293 genotypes of mungbean from the mungbean minicore set were cultivated in the stated four locations during different times, and the data for different parameters including the 100 SW were collected. The information regarding the locations, cropping duration and years are given in Table 1.

The genotypes were sown in three replications with five plants per replication. The plants were managed by following the management strategies in the “Suggested cultural practices for mungbean” provided by Lal *et al.* (2006). Data for 100 SW were collected by taking a sample of 100 seeds from the harvested plants randomly from each of the three replications. Genotypic data of the mungbean genotypes under study were obtained from the Dart PRL as designated by Breria *et al.* (2020). The imputation was also done in the genotypic data in order to make up for the missing data by using the LD KNNi imputation protocol (Money *et al.* 2015). The markers having more than 10% of the missing data along with and less than 5% of the minor allele frequency (MAF) after imputation were filtered out.

In order to determine the phylogeny and relationship among the genotypes along with finding out the number of sub-populations of the mungbean minicore set, an archaeopteryx tree was developed with Neighbour Joining clustering method developed by Saitou and Nei (1987) within TASSEL (Trait Analysis by Association, Evolution and Linkage) computer program. In order to develop the co-factor

for the GWAS, population structure was determined by the structure analysis in STRUCTURE computer program (version 2.3.4). The population structure provides deeper insights into the population subgroups (K) through the use of a Markov Chain Monte Carlo (MCMC) approach. The burn in period in the analysis was held to 50,000 and MCMC repeats were set to 500,000 for the K values from 2 to 15 (Pritchard *et al.* 2000; 2010).

The output of the analysis was analysed further by using the STRUCTURE HARVESTER online platform developed by Evanno *et al.* (2005) following the protocols of Earl and Vonholdt (2012). The results were supposed to provide the details of the population structure (K-value) of the minicore set under investigation. Finally, the GWAS was conducted using the TASSEL (version 5.0) computer program. The candidate SNP markers were manually examined in Microsoft Excel computer program by comparing the markers with change in phenotypic data of the 100 SW in the genotypes. The selected markers which were promising in association with 100 SW were selected and compared across the locations in order to find out the SNPs with highest probabilities to be associated with changes in 100 SW.

Results

The 100 SW data acquired from all of the four environments were compared. The average 100 SW of Pakistan, Bangladesh, Myanmar and Taiwan was 5.5, 3.6, 3.9 and 3.7 g, respectively. This indicated that seed weight on an average was highest in Pakistan and lowest in Bangladesh. Overall, the seed weight among the four locations was different, but correlated (Table 2; Fig. 1). The stability of 100 SW across the different environments was highly different among the studied genotypes.

The mungbean minicore set was genotyped and around 6,506 SNPs were identified over the 11 chromosomes of the genome. The analysis of population structure showed that the minicore set comprised of 3 sub-populations. The L(K) graph (Fig. 2A, B) indicated significant variation from the cluster 2 to the cluster 3 and the delta K (ΔK) graph on the other hand showed peak at the cluster 3 indicating that the mungbean genotypes minicore set belonged to 3 main clusters (Fig. 2C). It was also determined that the distribution of the seed weight was not even over the subpopulations. On an average, 100 SW of the mungbean was higher in case of the subpopulation 2 (average 5.9 g) as compared to the subpopulations 1 and 2 (average 3.8 g). The size differences between subpopulation 2 and the other two subpopulations was significant ($P < 0.001$).

The graph of the population structure was constructed by STRUCTURE computer program, which indicated the distribution of the studied mungbean genotypes under the respective sub-populations (Fig. 2). Apart from a few accessions, most of the genotypes showed some degree of admixture from the other genotypes among the sub-populations.

Table 1: Location information of the experiments

Country	Location	Coordinates	Cropping duration	Year
Pakistan	Islamabad	33°40'42.6" N, 73°08'20.4" E	Mar– May	2016
Bangladesh	Ishurdi	24°07'43" N, 89°03'57" E	Mar– May	2017
Myanmar	Yezin	19°50'02" N, 96°16'45" E	Nov– Jan	2016–17
Taiwan	Tainan	23°01'30" N, 120°17'35" E	Sep– Nov	2018

Table 2: Pearson correlation coefficients and *t*-test results of the comparison of 100 SW across four locations

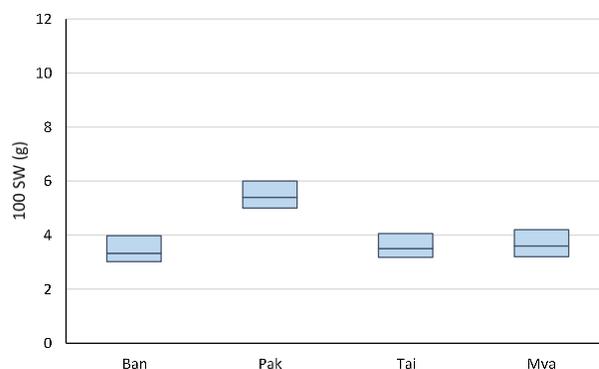
Statistical test	Pakistan	Taiwan	Myanmar
Pearson's	Bangladesh 0.49 (P < 0.001)	0.61 (P < 0.001)	0.55 (P < 0.001)
Correlation	Pakistan	0.62 (P < 0.001)	0.64 (P < 0.001)
Coefficient	Taiwan	-	0.82 (P < 0.001)
<i>t</i> -test	Pakistan	Taiwan	Myanmar
(<i>P</i> -value)	Bangladesh 7.58×10^{-83}	0.01	0.0003
		3.9×10^{-81}	2.53×10^{-55}

Table 3: Potential SNP positions associate with 100SW in mungbean

Chromosome	Pakistan	Bangladesh	Myanmar	Taiwan
1	-	-	-	25,049,038
	-	-	31,425,058	-
4	7,032,154	7,032,154	-	-
	11,559,392	-	-	-
6	-	5,433,578	-	-
	-	34,701,957	-	-
	-	34,712,555	-	-
	-	34,744,525	-	-
	-	34,913,611	-	-
	-	35,030,031	-	-
	36,662,459	-	-	-
	-	-	-	-
8	-	-	9,321,413	-
	-	-	9,379,508	-
	-	-	9,427,417	-
	-	-	9,427,833	-
	-	-	9,427,846	9,427,846
	-	-	9,432,667	9,432,667
	-	-	9,588,677	9,588,677
	27,458,129	-	-	-
	27,246,365	27,246,365	-	-
	-	28,480,139	-	-
-	28,480,205	-	-	
-	-	29,255,058	-	
-	-	35,992,362	-	
-	-	35,978,574	-	
9	9,495,582	-	-	-
	9,488,553	-	9,488,553	-

The population structure of the studied mungbean minicore set was in accordance with the phylogenetic tree developed in TASSEL software, which contained 3 major clusters of the genotypes, divided into the sub-clusters (Fig. 3). The quantile-quantile (QQ) plots from all of the locations presented various levels of association among the markers and the 100 SW. Results of the QQ plots indicated that the highest number of potential SNPs associated with the 100 SW were found among the minicore set cultivated in Myanmar (Fig. 4C), followed by Bangladesh (Fig. 4B), Pakistan (Fig. 4A) and Taiwan (Fig. 4D).

After the QQ plots, the Manhattan plots were constructed in order to locate the markers on their respective chromosomes which might be associated with


Fig. 1: 100 seed weight across multiple locations

the 100 SW (Fig. 5). The Manhattan plots of the association analysis in the genotypes cultivated in Pakistan indicated the potentially associated markers on the chromosomes 2, 4, 7, 8 and 9 (Fig. 5A) identifying the loci associated with the variation in 100 SW. On the other hand, in Bangladesh and Taiwan, the aligned SNPs were located on the chromosome 4, 6, 7 and 8 (Fig. 5B, D). Lastly, in Myanmar, the candidate SNPs were located on the chromosomes 7, 8 and 9 (Fig. 5C).

After the selection of the aligned markers above the threshold, the link between the SNPs and variation in 100 SW was determined by aligning the candidate SNPs with 100 SW and the genotypes in the ascending order. This was beneficial in weeding out the false positive markers and choosing the markers that were somewhat linked with the variation in 100 SW. Table 2 demonstrates that chromosome 8 is home to the majority of the associate loci. Additionally, some of the sites are discovered commonly to be associated to 100 SW in more than one location. This indicated that the gene(s) responsible for the 100 SW may be found near these locations of the markers on chromosomes 4 and 8. It was found that some of the markers were associated with the 100 SW in a particular environment, while some were found to be common across the locations. This indicated that some sites were influenced by the environment while others were not. Some loci on chromosomes 4 and 8 were found to be potentially associated with 100 SW in Pakistan and Bangladesh, and some were common in Myanmar and Taiwan (Table 3).

Discussion

In this study, the correlation among the seed weight across the four locations indicated that the seed weight is correlated across the environments which means that the genetic effects of the seed weight are stronger than the environment and the environment only modifies the effects of the genes in expressing the 100 SW up to an extent (Kato *et al.* 2014). This finding was supported by the previous investigations of Yimram *et al.* (2009) who found that 100 SW in studied mungbean genotypes had genotypic variance of 1.64 and environmental variance of 0.14. In addition to this, the phenotypic coefficient of variation was observed to

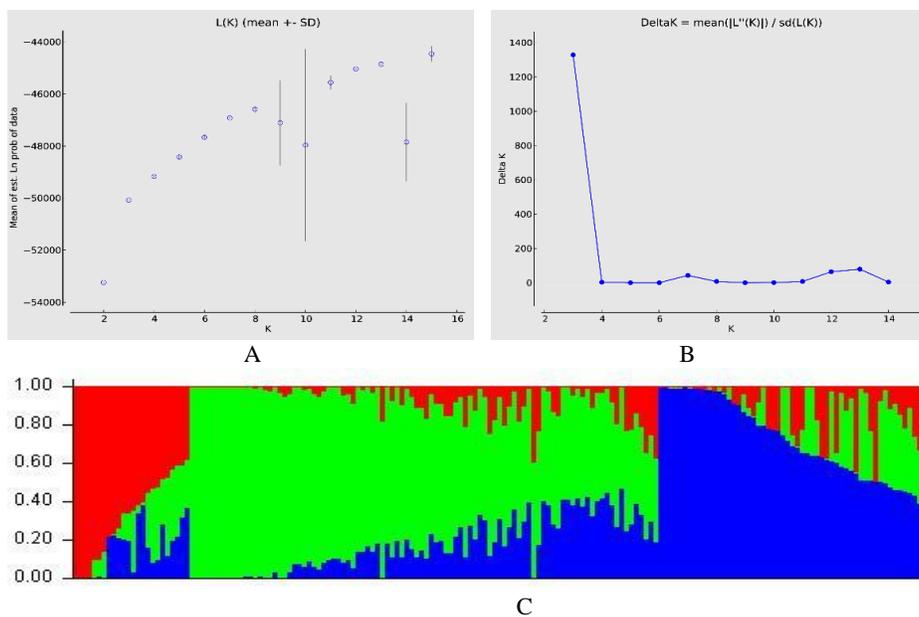


Fig. 2: STRUCTURE HARVESTER mediated results for number of possible sub-populations and population structure of the studied genotypes

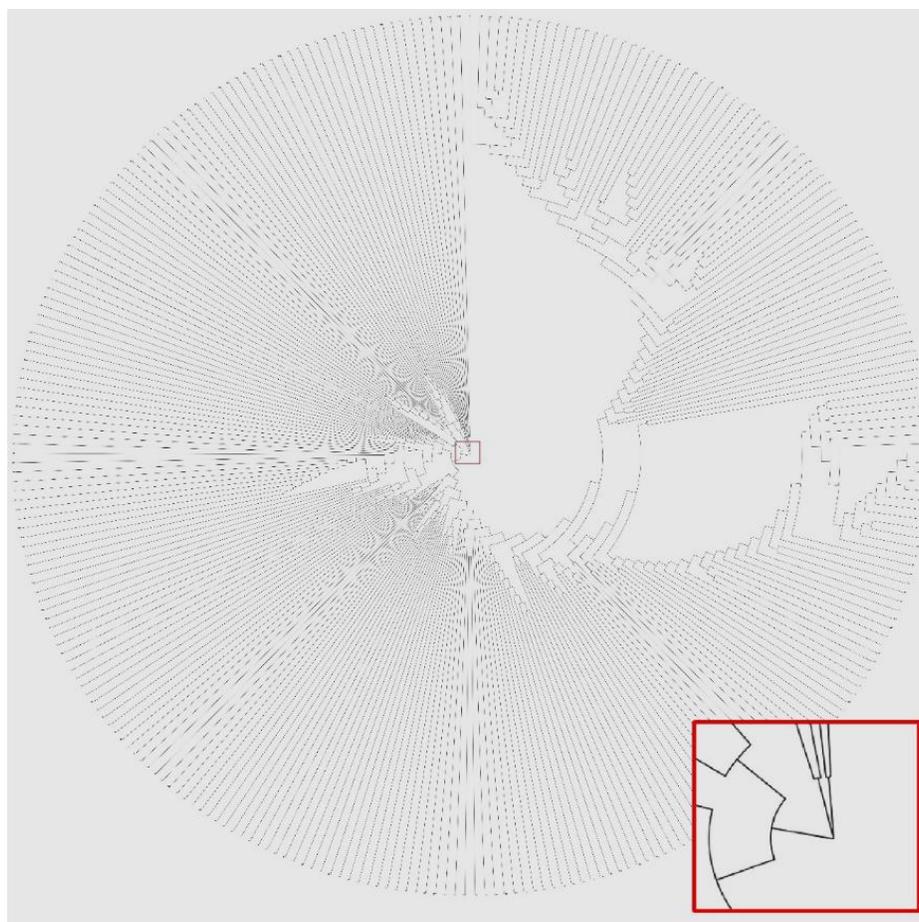


Fig. 3: Arch tree of the studied minicore genotypes

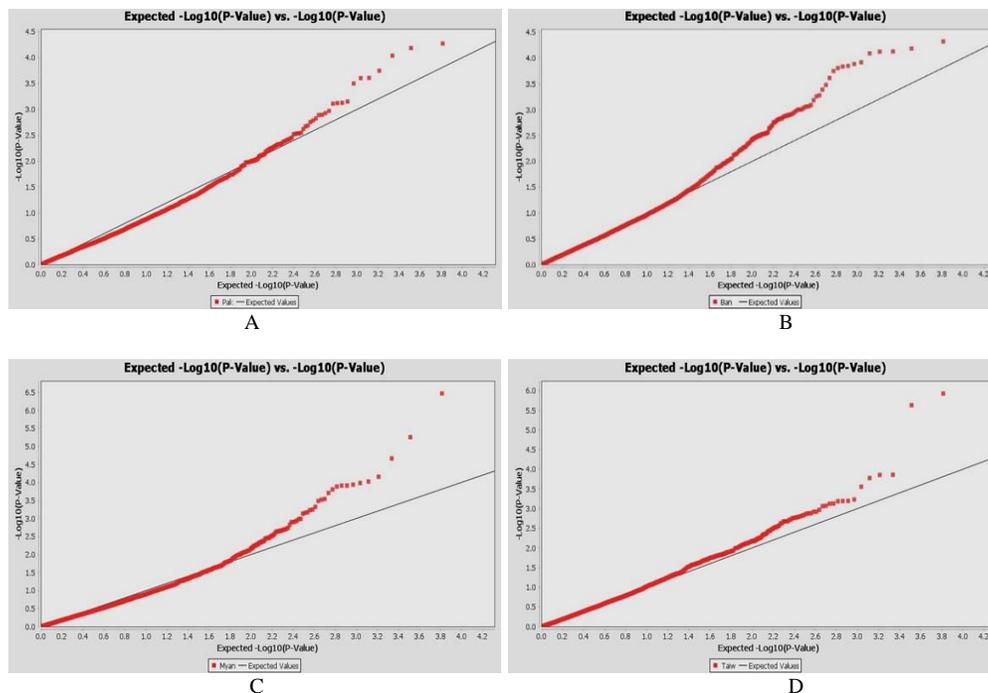


Fig. 4: Quantile-quantile (QQ) plots for 100 seed weight of studied mungbean minicore set cultivated in Pakistan (A), Bangladesh (B), Myanmar (C) and Taiwan (D)

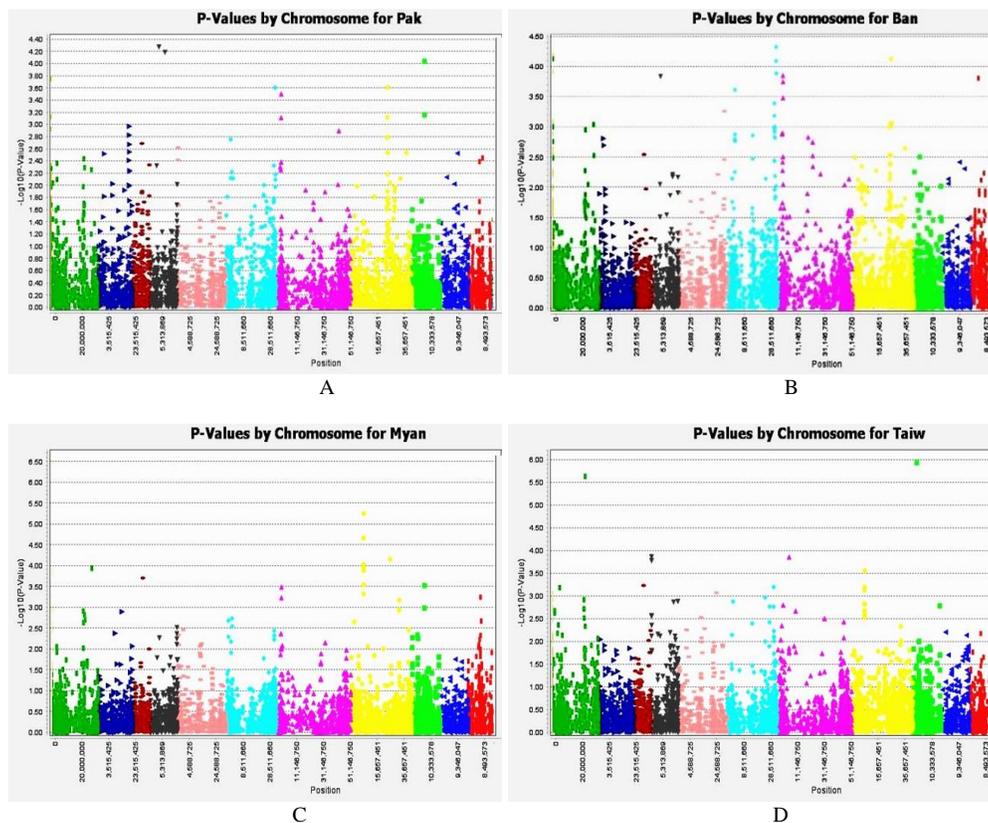


Fig. 5: Manhattan plots for 100 seed weight of studied mungbean minicore set cultivated in Pakistan (A), Bangladesh (B), Myanmar (C) and Taiwan (D)

be 29.40 and genotypic coefficient of variation was 28.21, which means that almost all of the variation among the 100 SW is because of genetic variation (Baye 2002). In this regard, Kumar *et al.* (2010) showed that because of this property, the seed weight is a suitable parameter for the GWAS.

Regarding the GWAS, the population structure of the studied mungbean minicore set belonged to 3 sub-populations, which indicated that the genetic attributes of the minicore genotypes belonged to three characteristic sets. Among the different parameters, the parameter of 100 SW was different in case of the sub-population 2 as compared to the sub-populations 1 and 3. In contrast to the findings, Sokolkova *et al.* (2020) determined that the WorldVeg minicore set belonged to 4 sub-populations. On the other hand, Breria *et al.* (2020) determined that the minicore set belongs to 2 to 4 sub-populations which indicates that it is possible for the minicore set to belong to 3 sub-populations.

The QQ graphs plot the implication of the associated SNPs with the character under investigation. The line having upwards inclined slope indicates the expected probability line of markers' association undertaking that there is no significant association of the markers with the studied parameter among the studied genotypes. The dots around the inclined line however show the actual measured probabilities of the markers' association with parameter (Armour *et al.* 2014). The deviance of the dots from the inclined line indicates the presence of some association among the markers and 100 SW, and the distance of the deviance shows the strength of the association among the markers and the parameter. The bigger the distance is among the expected and measured probability, the stronger the association will be, and vice versa (Kang *et al.* 2015). The deviation of the actual probability of the markers' association with the 100 SW from the expected probability indicated that there are some markers associated with the 100 SW in the studied mungbean minicore set (Fig. 4). The highest number of markers possibly associated with the 100 SW were found in Myanmar, followed by Bangladesh, Pakistan and Taiwan (Table 3). The results of QQ plots (Fig. 4) and selected markers potentially associated with 100 SW (Table 3) were correlated. This indicates that if there are more markers deviating from expected probabilities in QQ plots, the number of potential markers selected for association to a trait will be more (Wang and Zhang 2021). The Manhattan plots were constructed in order to determine the association and probability of the SNP positions with the 100 SW in mungbean. It can be observed that the random dispersal of the SNP markers over every chromosome indicates that there is no significant association among the markers and 100 SW (Ahmed *et al.* 2021). On the other hand, the markers which are located in a straight line above a specific limit such as 4.0 (-log₁₀) show significant association with 100 SW. The alignment of the associated markers is developed in a straight line if the associated SNPs are located nearby or within a single gene controlling the

trait. This simplifies the identification of the candidate genes being associated in the variation of the trait of interest (Hussain *et al.* 2018). In the four locations, the markers potentially associated with the 100 SW were located on the chromosomes 1, 2, 4, 6, 7, 8 and 9 which indicate that the markers associated with the 100 SW are highly probably located on one or more of the mentioned chromosomes.

In order to determine whether there is any real association of the selected SNPs with the 100 SW, the data for the 100 SW of all the genotypes were arranged in ascending order along with the respective SNP at that location on Microsoft Excel in order to visualize if the change in the marker is actually related to the change in the 100 SW as suggested by Tian *et al.* (2011). This alignment of the SNPs with the 100 SW in ascending order further screened out fewer SNPs, which were responsible for the change in the 100 SW in the genotypes as indicated by Dereeper *et al.* (2011). The markers located on the chromosomes 1, 4, 6, 8 and 9 were shortlisted as the potential markers to be associated with the 100 SW after the alignment analysis. Genetic analysis of the domestication-related parameters in the mungbean population which was derived from the wild genotype "JP211874" in Myanmar and another mungbean genotype "Sukhothai" also indicated some loci on the chromosome 8 which corresponded to the chromosome 8 of the provided reference sequence of Kang *et al.* (2014) and Kang *et al.* (2015) between markers "CEDG059 (position ~ 15,462,360 bp) and VM37 (position ~ 29,308,079)" (Isemura *et al.* 2012). These loci were found to be overlapping with the candidate markers found associated with the 100 seed weight in Pakistan and Bangladesh. The seed weight related markers mapped by Alam *et al.* (2014) could not be located on any of the chromosomes, however the physical location of those markers were also somewhat similar to the one found in the present investigation. It was found that some of the markers were associated with the 100 SW in a particular environment, while some were found to be common across the locations. This indicated that some sites were influenced by the environment while others were not. Most importantly, the markers located on the chromosomes 4 and 8 were found to be common across different environments to be associated with the 100 SW which indicates that these markers have high probability to be associated with 100 SW. Therefore, these markers can be studied further in order to determine their relation with the genes causing the change in 100 SW in mungbean.

Conclusion

The mungbean minicore set comprised of three sub-populations. On chromosome 8, there are a majority of the markers potentially associated to 100 seed weight in mungbean. In trials conducted in Pakistan and Bangladesh, two SNPs "4:7,032,154 and 8:27,246,365" were found to be common, which could be associated with 100 SW in addition

to locally associate markers. On the other hand, the trials conducted in Myanmar and Taiwan resulted in finding three markers “8:9,588,667, 8:9,427,846 and 8:9,432,667” to be common and potentially associated with 100 SW. These SNPs are probably associated to 100 SW in mungbean, however more research and validation are required in order to further establish these findings.

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Author Contributions

MAA conducted the research and performed the analyses, MA supervised the study and contributed in planning, RS supervised the analyses and assisted in write-up, RMA assisted in planning and data collection, and GM contributed in analysing the data and developing understanding

Conflict of Interest

Authors declare no conflict of interests

Data Availability

We hereby declare that the data related to this article, are available with the corresponding author and will be provided on request

Ethics Approval

Ethical approval is not applicable in this study

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