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Genome-Wide Association Study of Complex Traits in Maize Detects Genomic Regions and Genes for Increasing Grain Yield and Grain Quality

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Abstract. This review describes the current status of genome-wide association study (GWAS) of the major crops in maize (Zea mays L.) concentrate on performing association mapping as a novel method in associating genetic and complex traits, current strategy in analyzing of phenotype and genotype data to identify population structure and linkage disequilibrium. GWAS has an important role in food security because this method identified many crucial genomic regions of important traits in the most commercialize crops of the world, such as maize. These complex traits including yield, grain quality, biofortification, biotic and abiotic resistance. GWAS has many advantages correlated with reducing genotyping cost and research time, increasing mapping resolution and larger allele number. Meanwhile, GWAS has two main limitations related to population size and the number of markers. There are many software packages for data analysis in GWAS. The most commonly software that was used in GWAS especially in this crop is TASSEL because frequently updated. Recently, many research papers concentrated on GWAS in maize. GWAS analysis accelerated identification of genetic regions, candidate genes within these genomic regions and their metabolomic analysis correlated to the complex traits in maize for increasing grain yield and grain quality to fulfill the market demands.

Keywords: complex traits, genomic regions, GWAS, maize, grain yield, grain quality

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1. Introduction

Genome-wide association study (GWAS) identified variation of the genetic characteristics within the genome in order to analyze correlation of the complex traits [1,2,3]. GWAS increases the acceleration and accuracy of locating quantitative loci and candidate genes that can be used for crop improvement. Functional analysis of these candidate genes will enhance our understanding related to the crop response mechanism to the environments. This mapping is very affordable to plant research programs because of the development in genomic technology and statistical analysis methods [4]. Population of the maize for GWAS have to contain optimum sample size that show population structure and kinship relationships with diverse genetic variation of the complex traits [5,6]. Compared to QTL analysis, GWAS can identify many alleles at the same spot and detect genes associated with the complex traits with higher resolution [7,8]. Thus, GWAS is an effective way to identify the genomic regions that regulate the complex traits in maize. For example, seed and grain morphology, plant morphology, plant development (flowering time), yield, abiotic and biotic stress, cooking, eating and nutritional quality-related traits that correlated with environmental variations.

In 2005, GWAS was used for the first time to detect the relationship between age and molecular degeneration. Right now, GWAS have been widely used to detect the genomic regions correlated with the complex traits in human, animals, and plants. In plants, GWAS have been detected many genes associated to the complex traits, such as in Arabidopsis, rice, maize, sorghum, wheat, barley, and many other plants [2,9-22]. Phenotypic data and genome-wide genotypes are required in GWAS. GWAS simultaneously screened large number of genetic accessions. The power of the GWAS can be enhanced by using a large number of sample size to increase the variation of the genetic characteristics of the complex traits. GWAS became a new method for identification genes related to the important traits influenced by the rapid development in the sequencing techniques, such as next-generation sequencing that can sequence faster, better, and cheaper compared to the previous sequencing techniques. The design of the GWAS consists of population structure identification, case subject selection, control subject selection, and genotyping a million single nucleotide polymorphisms (SNPs). Recently, SNPs has been using widely due to their density within the genome, higher resolution in mapping, and more cost effective [1]. GWAS has many advantages in reducing genotyping cost and research time, increasing mapping resolution and larger allele number. Several challenges in GWAS that related to multiple hypothesis testing, population structure and statistical power and resolution [23,24,25].

Several statistical methods can be applied to reduce false positive due to population structure and kinship. Various software packages for data analysis in GWAS. The most commonly software that was used in GWAS especially in maize is TASSEL because frequently updated [26,27]. For advanced researchers usually used SAS software [28] or R [29] because it requires programming skills to develop various methods. STRUCTURE software is used to estimate population structure [30]. Kinship relationship among the samples can be estimated by using SPAGeDi [31]. The principal components analysis can be analyzed with EINGENSTRAT software [32]. PowerMarker is used to detect genetic distance among the accessions.

High-resolution genetic mapping in GWAS enhance the accuracy of the candidate genes and identify novel genes related to the important traits. GWAS plays an important role in maize breeding programs. Hundreds of genes correlated with important traits have been clone and targeted genome editing with increasing accuracy and resolution [33-40]. This review describes the current status of GWAS in maize concentrate on performing association mapping as a novel method in associating genetic and complex traits, current strategy in analyzing of phenotype and genotype data to identify population structure and linkage disequilibrium.

2. Methods

The literature studies, published between 1996 and 2022 were collected from the main International data bases, including PubMed, Scopus, and Web of Science. The following keywords were used in the title and abstracts, such as “maize” OR “GWAS” OR “complex traits” OR “genomics regions” OR “grain yield” OR “grain quality” OR “genes”. The reference list in the published studies was also checked to identify more relevant studies. Articles from the literature search were identified and selected based on the selection criteria, including (a) full-text published articles in the English language; (b) studies that have reported the GWAS in maize. The required information that was found from all eligible articles was as follows: the year of publications, population of study, sample size, background markers, complex traits, loci, and chromosome.

3. Results and Discussion

In maize, GWAS conducted in 300 maize inbred lines [41]. Based on the carotenoid content trait, GWAS identified genomic region lycopene epsilon cyclase (lcyE) correlated with carotenoid biosynthetic pathway in diverse maize inbred lines [42]. Furthermore, the correlation between beta-carotene and grain color is low, so screening and selection to produce maize with high vitamin A level is more effective based on lcyE alleles than grain color. This research is very useful in high provitamin A maize breeding program. According to Wang et al. [24], GWAS is a crucial method to identify genetic factors related to head smut resistance in maize that causes reduction in quality and yield of maize every year. In this research, used Illumina MaizeSNP50 array, 45,868 SNPs and 144 inbred lines maize. GWAS identified 18 candidate genes correlated with head smut resistance that very important to develop head smut-resistant maize cultivars.

Based on Tian et al. [10], 1.6 million SNPs and 4,892 diverse lines were used for GWAS of leaf architecture, such as leaf length, width, and angle that associated with the light capture efficiency. Furthermore, maize yield will increase as the light capture become more efficient. GWAS also identified Dwarf8 (D8) gene that regulated flowering time in maize by using 375 maize inbred lines and 275 maize landraces with 55 simple sequence repeat (SSR) markers [43,44,45]. In Belo et al. [46], GWAS detected genomic regions on chromosome 4 that controlled oleic acid content in maize using 553 maize inbred lines and 8,590 SNPs.

GWAS in husk tightness of maize was identified by Jiang et al. [47] that very useful for genetic improvement related to the protection of maize kernel from pathogen and pest, and also mechanical damage in harvesting process. In this study used 508 maize inbred lines. Based on the phenotypic analysis, maize from temperate environments showed more loosely compared to tropical and subtropical environments. This study identified 27 candidate genes by using husk tightness phenotypic data and ~1.25 million SNPs. These candidate genes have important functions in husk senescence, morphogenesis, and abiotic stress defense.

In 2021, Zheng et al. [48] identified 49 loci on chromosome 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 associated with grain quality of the maize by GWAS analysis. These grain quality traits, including moisture, protein, oil, starch, and lysine contents. A total of twenty-nine candidate genes identified within the loci that correlated to biological processes, cellular components, and molecular functions. These results provide an important strategy to develop high-quality varieties in maize breeding program. A total of 63 loci and 189 candidate genes associated with root system architecture in maize were identified by Wu et al. [22] using GWAS analysis. This GWAS analysis performed by using 1.25 million SNPs and 421 maize inbred lines. The results of this GWAS analysis are important to develop maize varieties with improved root systems. Many research papers were published related to GWAS analysis in maize from 2011 until 2022 (see Table 1).

Table 1: Genome wide association studies in maize (*Zea mays*)

Year	Population	Sample Size	Background Markers	Traits	Loci	Chromosome	References
2011	Diverse inbred lines	4,892	1.6 million SNPs	Leaf architecture (leaf length, leaf width and upper leaf angle)	-	2 and 3	(Tian et al., 2011) [10]
	Recombinant inbred lines	5,000	1.6 million SNPs	Southern leaf blight disease	32	1, 2, 3, 4, 5, 6, 7, 8, 9, and 10	(Kump et al., 2011) [49]
	Inbred-line nested association	5,000	1.6 million SNPs	Northern leaf blight resistance	29	1, 2, 3, 4, 5, 6, 7, 8, 9, and 10	(Poland et al., 2011) [50]
	Diverse inbred lines	284	55,000 SNPs	Plant height	105	5	(Weng et al., 2011) [51]
2012	Diverse inbred lines	144	1.6 million	Head smut resistance	19	1, 2, 3, 4, 5, 6, 7, 8, 9, and 10	(Wang et al., 2012) [24]
	Diverse inbred lines	289	56,110 SNPs	Leaf metabolic profiles	-	1, 3, 4, 5, 6, 9, and 10	(Riedelsheimer et al., 2012) [11]

	Diverse inbred lines	543	56,110 SNPs	a-tocopherol content	-	2, 4, and 5	(Li et al., 2012) [52]
2013	Diverse inbred lines	368	1.03 million SNPs	Kernel oil concentration and fatty acid composition	74	1, 2, 3, 4, 5, 6, 7, 8, and 10	(Li et al., 2013) [53]
	Diverse inbred lines	267	47,445 SNPs	Fusarium ear rot resistance	3	1, 5, and 9	(Zila et al., 2013) [54]
	Diverse inbred lines	281	591,822 SNPs	Tocochromanol levels in maize grain	16	5	(Lipka et al., 2013) [55]
	Diverse inbred lines	284	39,166 SNPs	Flowering time, kernel composition, and disease resistance	46	1, 2, 3, 4, 5, 6, 7, 8, 9, and 10	(Schaefer et al., 2013) [56]
	Diverse inbred lines	350	44,314 SNPs	Grain yield and related phenotypic traits under drought stress:			(Xue et al., 2013) [57]
				- Grain yield (GY)	6	1, 8, 9, and 10	
				- Hundred kernel weight (HKW)	3	1, 8, and 9	
				- Kernel number (KNO)	2	5 and 9	
				- Ear height (EH)	10	1, 4, 6, and 9	
				- Plant height (PH)	4	1, 2, 3, and 10	
				- Relative ear position (EPO)	11	1, 3, 4, 6, and 10	
				- Female flowering, (days to silk DTS)	2	4	
				- Male flowering, (days to anthesis DTA)	4	1, 8, and 9	
				- Anthesis-silking interval (ASI)	5	1, 3,and 9	

Table 2: Genome wide association studies in maize (*Zea mays*) (*continued*)

Year	**Population**	**Sample Size**	**Background Markers**	**Traits**	**Loci**	**Chromosome**	**References**
2014	Diverse inbred lines	368	56,110 SNPs	Metabolites	1459	1, 2, 8, and 9	(Wen et al., 2014) [58]
	Diverse inbred lines	281	462,702 SNPs	Carotenoid levels	8	6, 8, 9, and 10	(Owens et al. 2014) [59]
	Diverse inbred lines	3,381	26.5 million SNPs	Hypersensitive defense response	32	1, 2, 3, 4, 5, 6, 8, 9, and 10	(Olukolu et al., 2014) [60]
	Diverse inbred lines	513	556,809 SNPs	17 agronomic traits:	34		(Yang et al., 2014) [37]
				-Plant height		1, 2, 3, 4, 5, 9, and 10	
				-Ear height		1, 2, 3, 4, 5, 6, 8, 9, and 10	
				-Ear leaf width		2, 3, 4, 5, 7, 8, 9, and 10	
				-Ear leaf length		2, 3, and 9	
				-Tassel main axis length		2, 3, 4, 5, 7, and 10	
				-Tassel branch number		2, 4, 5, 6, 7, 8, 9, and 10	
				-Leaf number above ear		2, 3, and 9	
				-Ear length		1, 2, 3, 5, 7, 9, and 10	
				-Ear diameter		1, 3, 4, 5, 7, 9, and 10	
				-Cob diameter		1, 2, 3, and 5	

				-Kernel number per row		1, 5, 6, 8, and 9	
				-100-grain weight		1 and 9	
				-Cob weight		2	
				-Kernel width		1, 2, 5, and 7	
				-Days to anthesis		1, 2, 3, 4, 5, 6, 7, and 8	
				-Days to silking		1, 3, 4, 5, 6, 7, and 10	
				-Days to heading		1, 2, 4, 5, and 10	
2015	Diverse inbred lines	384	681,257 SNPs	Seedling root development	268	1, 2, 3, 4, 5, 6, 7, 8, 9, and 10	(Pace et al., 2015) [61]
	Diverse inbred lines	615	2,000 SNPs	Lethal necrosis disease resistance	24	1, 2, 3, 4, 5, 6, 7, 8, 9, and 10	(Gowda et al., 2015) [62]
	Diverse inbred lines	300	261,184 SNPs	*Aspergillus flavus* and Aflatoxin Accumulation Resistance	107	1, 2, 3, 4, 5, 6, 7, 8, 9, and 10	(Warburton et al., 2015) [63]
	Diverse inbred lines	5,000	1.6 million SNPs	Carbon and Nitrogen Metabolism	-	1, 2, 3, 4, 5, 6, 7, 8, 9, and 10	(Zhang et al., 2015) [64]

Table 2: Genome wide association studies in maize (*Zea mays*) (*continued*)

Year	**Population**	**Sample Size**	**Background Markers**	**Traits**	**Loci**	**Chromosome**	**References**
2016	Diverse inbred lines	367	525,104 SNPs	Drought tolerance	83	1, 2, 4, 7, 8, 9, and 10	(Wang et al., 2016) [65]
	Diverse inbred lines	508	543,641 SNPs	Genetic architecture of four husk traits	9	4, 5, 6, 8, 9, and 10	(Cui et al., 2016) [66]
	Diverse inbred lines	368	559,285 SNPs	Stalk cell wall components: lignin (LIG), cellulose (CEL) and hemicellulose (HC)	64	1, 2, 3, 4, 5, 6, 7, 8, 9, and 10	(Li et al., 2016) [67]
	Diverse inbred lines	274	246,497 SNPs	Common rust resistance	3	2, 3, and 8	(Olukolu et al., 2016) [68]
	Diverse inbred lines	318	156,599 SNPs	Drought-related metabolic changes:	63		(Zhang et al., 2016) [69]
				-Sucrose (Suc)		1, 2, 2, 5, 5, 5, 6, and 10	
				- Total sugars (Tsug)		1, 1, 2, 2, 2, 3, 4, and 6	
				- Phaseic acid (Pa)		1, 1, 1, 3, 3, 4, 4, 4, 5, 5, 6, 6, 6, 6, 7, 7, and 7	
				- Glucose (Glc)		1, 1, 2, 3, 4, 4, 5, 6, 6, and 6	
				- Abscisic acid glucose ester (ABA-GE)		1, 1, 2, 2, 4, 4, 6, and 6	
				- Proline (Pro)		1, 1	
				- Starch (Str)		2, 10, and 10	
				- Abscisic acid (ABA)		2, 3, 3, 3, 5, 5, 8, 9, and 10	
				- Specific leaf weight (Slw)		5 and 7	
				- Physiological traits including dry mass (Dw)		6 and 7	
2017				Review GWAS from 2011 until 2016			(Xiao et al., 2017) [70]

2018	Diverse inbred lines	292	25,331 SNPs	Major ear quantitative traits	20	1, 2, 3, 4, 5, 6, 7, 8, 9, and 10	(Zhu et al., 2018) [71]
	Diverse inbred lines	157	355,972 SNPs	Gray leaf spot resistance	7	1, 2, 3, 4, 6, 7, and 10	(Kuki et al., 2018) [72]
	Diverse inbred lines	287	260,550 SNPs	Corn earworm resistance	51	1, 2, 3, 4, 5, 6, 7, 8, 9, and 10	(Warburton et al., 2018) [73]
	Diverse inbred lines	300	62,077 SNPs	Root system architecture traits	19	1, 2, 5, 7, and 8	(Sanchez et al., 2018) [18]
2019	Diverse inbred lines	356	541,575 SNPs	13 traits in maize seedlings under low phosphorus stress	551	1, 2, 3, 4, 5, 6, 7, 8, 9, and 10	(Wang et al., 2019) [74]
	Diverse inbred lines	555	681,257 SNPs	Goss's wilt resistance	10	1, 2, and 5	(Singh et al., 2019) [75]
	Diverse inbred lines	282	39,991 SNPs	Metabolites	31	1, 2, 3, 4, 5, 6, 7, 8, 9, and 10	(Zhou et al., 2019) [76]

Table 2: Genome wide association studies in maize (*Zea mays*) (*continued*)

Year	**Population**	**Sample Size**	**Background Markers**	**Traits**	**Loci**	**Chromosome**	**References**
2020	Diverse accessions	419	955,690 SNPs	Northern corn leaf blight resistance	17	1, 2, 3, 4, 5, 6, 7, 8, 9, and 10	(Rashid et al., 2020) [20]
	Diverse inbred lines	639	42,667 SNPs	Kernel row number	7	1, 2, 3, 5, 9, and 10	(An et al., 2020) [77]
	Diverse inbred lines	410	7,490 SNPs	Gray leaf spot resistance	22	1, 2, 6, 7, and 8	(Kibe et al., 2020) [78]
2021	Diverse inbred lines	412	779,855 SNPs	Aboveground Dry Matter Accumulation at Seedling Stage	678	1, 2, 3, 4, 5, 6, 7, 8, 9, and 10	(Lu et al., 2021) [79]
	Diverse inbred lines	281	1.25 million SNPs	Genetic architecture of root hair length	11	1, 2, 4, 5, 6, and 10	(Liu et al., 2021) [80]
	Diverse accessions	424	955,690 SNPs	Stover quality traits	12	1, 2, 3, 5, 6, and 7	(Vinayan et al., 2021) [81]
	Diverse inbred lines	179	1,490,007 SNPs	Root architectural traits at multiple seedling stages	8	1, 2, 4, and 10	(Moussa et al., 2021) [82]
	Diverse inbred lines	248	83,057 SNPs	Grain quality traits	49	1, 2, 3, 4, 5, 6, 7, 8, 9, and 10	(Zheng et al., 2021) [48]
2022	Diverse inbred lines	421	1.25 million SNPs	Root system architecture	63	1, 2, 3, 4, 5, 6, 7, 8, 9, and 10	(Wu et al., 2022) [22]

GWAS analysis reported accurate loci and candidate genes related to many complex traits in maize, including seed development, phenotypic plant morphology, plant development, yield, kernel quality, abiotic and biotic stress-related traits [83-92]. These research papers provide important information to enhance maize breeding program that develop improved maize varieties in grain yield and the quality.

4. Conclusion

GWAS analysis accelerated identification of genetic regions, putative genes, and various metabolic pathways correlated with important economic traits in maize to fulfill the market demands. These traits are related with grain yield and grain quality that supported by agronomic traits, biotic and abiotic stress resistance. GWAS has many advantages in reducing genotyping cost and research time, increasing mapping resolution and larger allele number. Development of the software package for GWAS determined accelerating improvement of the maize characteristics.

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