

# Characterization of a major QTL and Genome-Wide Epistatic Interactions for the Transformation of Single Spikelet in Teosinte Ears into Paired Spikelets in Maize Ears During Maize Domestication

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## Research Article

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## Abstract

Maize ear carries paired spikelets, whereas the ear of its wild ancestor, teosinte, bears single spikelets. However, little is known about the genetic basis of the processes of transformation of single spikelets in teosinte ear to paired spikelets in maize ear. In this study, a two-ranked, paired-spikelets primitive maize and a two-ranked, single-spikelet teosinte were utilized to develop an F<sub>2</sub> population, and QTL mapping for single vs. paired spikelets (PEDS) was performed. Two QTL (*qPEDS1.1* and *qPEDS3.1*) for PEDS located on chromosomes 1L and 3S were identified in the 162 F<sub>2</sub> plants using the inclusive composite interval mapping of additive (ICIM-ADD) module, explaining 1.93% and 23.79% of the phenotypic variance, respectively. Out of the 409 F<sub>2</sub> plants, 43 plants with PEDS = 0% and 43 plants with PEDS > 20% were selected for selective genotyping; the QTL (*qPEDS3.1*) accounting for 64.01% of the phenotypic variance for PEDS was also detected. Moreover, the QTL (*qPEDS3.1*) was validated in three environments, which explained 31.05%, 38.94% and 23.16% of the phenotypic variance, respectively. In addition, 50 epistatic QTLs were detected in 162 F<sub>2</sub> plants using the two-locus epistatic QTL (ICIM-EPI) module; they were distributed on all 10 chromosomes and explained 94.40% of the total phenotypic variance. The results contribute to a better understanding of the genetic basis of domestication of paired spikelets and provide a genetic resource for future map-based cloning; in addition, the systematic dissection of epistatic interactions underlies a theoretical framework for overcoming epistatic effects on QTL fine mapping.

## Introduction

During maize domestication, the main goals were to improve the yield and ease of harvesting (Wang et al. 2005; Doebley 2004). The morphology of maize ear and that of its ancestral teosinte exhibit extreme differences, with taxonomists classifying them in different genera initially (Doebley 2004). There are four major traits distinguishing teosinte ear from maize ear (Doebley 1992, 2004). First, compared to the teosinte kernels protected by a hard outer glume, the maize kernels are naked, enabling the kernels to be easily harvested and digested. Second, the teosinte ear has two ranks with one rank on each side, whereas the maize ear has two or more ranks, resulting in maize ear bearing hundreds of kernels. Third, teosinte has a shattering rachis and maize has a non-shattering one; loss of shattering facilitates easy collection of kernels, and has been selected for in domestication of all cereals. Fourth, each cupulate fruitcase of the teosinte ear has single mature spikelet (one kernel per spikelet), whereas paired mature spikelets are in each cupulate fruitcase of the maize ear, which could double the kernel number. In addition to the single vs. paired spikelets, the key genes affecting the other three traits have been cloned, including *Teosinte glume architecture 1 (tga1)* involved in the origin of naked kernels (Dorweiler et al. 1993; Wang et al. 2005); *ZmSh1-1*, *ZmSh1-5.1* and *ZmSh1-5.2* related to seed shattering (Lin et al. 2012); and *FLORICAULA/LEAFY* homologs *zfl1* and *zfl2*, *unbranched2 (ub2)* and *unbranched3 (ub3)* and its regulatory locus *KRN4* responsible for rank number of ear determining number of kernel rows (Bombliès et al. 2003; Bombliès and Doebley 2006).

Observing the development of the female inflorescence by scanning electron microscopy, it was found that the transformation of the spikelet pair meristems (SPM) to the spikelet meristems (SM) was the crucial step involved in single vs. paired spikelets. In maize, the SPM produced two morphologically distinct spikelet meristems, one sessile and one pedicellate. Both of them developed into floral meristems (FM) that ultimately produced the floral organs (Upadyayula et al. 2006). In contrast, the pedicellate SM was aborted in teosinte (Doebley et al. 1995b). In the past 100 years, few studies were conducted to dissect the genetic basis of single vs. paired spikelets through analysis of Mendelian inheritance and linkages with a known gene or molecular marker loci. Colins and Kempton (1920) first reported that a ratio of paired:single spikelets approaching 3:1 was obtained in teosinte-maize hybrid populations, but the inheritance of single vs. paired spikelets deviated from the Mendelian pattern due to continuous phenotypic variation. Conversely, Langham (1940) inferred that single spikelet (named *pd*) was controlled by a single Mendelian factor. Mangelsdorf (1947) and Szabó et al. (1996) suggested that the segregation of single vs. paired spikelets fitted a model with two independent genes. Nevertheless, Rogers (1950) found that unifactorial inheritance did not control the single vs. paired spikelets, and the genetic control of the trait was more complex. Mangelsdorf (1947) showed the molecular marker loci on chromosomes 1L, 2S, 3L, and 4S link with single vs. paired spikelets. Subsequently, with the development of molecular marker technology, a RFLP marker was used for mapping QTL for single vs. paired spikelets. A total of 18 QTL for single vs. paired spikelets were identified, being located on all 10 chromosomes. The phenotypic variation explained by individual QTL ranged from 4.9–46.1% (Doebley et al. 1990; Doebley and Stec 1991, 1993; Lauter and Doebley 2002b). Two major QTL located on chromosomes 1L (QTL-1L) and 3L (QTL-3L) were detected in three different genetic backgrounds and the effect of these two QTL was confirmed by analyzing of near-isogenic lines (NILs) (Doebley et al. 1995a).

Epistasis is used to denote the interaction between alleles from different loci and plays an important role in quantitative genetics analysis (Mackay 2014; Vazquez et al. 2015; Shang et al. 2016). Recently, epistasis was reliably detected to have an effect on complex trait variation, including disease resistance (Roncallo et al. 2012; Singh. *et al*, 2013; Vazquez et al. 2015) and yield-related traits (Jiang et al. 2017; Soyk et al. 2017; Sundaram et al. 2018). Based on several experiments in which the total phenotypic variance of epistasis ranged

from 16–81%, Carlborg and Haley argued that epistasis should be accounted for in order to understand the genetic basis of complex traits (Carlborg and Haley 2004). In previous studies, the significant epistasis between loci *umc107* (QTL-1L) and *umc92* (QTL-3L) for single vs. paired spikelets was detected in the maize × teosinte (*Zea mays* ssp. *mexicana*) F<sub>2</sub> population (Doebley and Stec 1991). In the maize × teosinte (*Zea mays* ssp. *parviglumis*) F<sub>2</sub> population, the proportion of paired spikelets increased from 0–60% due to the combined effect of these two QTL (Doebley et al. 1995a). Then, the near-isogenic lines (NILs) of QTL-1L and QTL-3L were constructed in the maize and teosinte genetic backgrounds, respectively. The mean trait values of maize allele of QTL-1L and QTL-3L in the teosinte genetic background were 0.8% and 1.9%, respectively; the value was 7.3% when these two QTL were combined, which was 4.6% higher than the sum of two individual QTL. In addition, compared with the combined effect of these two QTL in the F<sub>2</sub> population (60%), about 52.7% reduction was observed in the teosinte genetic background, suggesting that the higher-order epistatic interactions may be responsible for PEDS (Doebley et al. 1995a).

In this study, a two-ranked (four rows) primitive maize was chosen to develop an F<sub>2</sub> population by crossing it with two-ranked, two-rows teosinte (*Z. mays* ssp. *mexicana*). The oligonucleotides pool assay (OPA) with 3072 well-distributed and high-quality SNPs was used for genotyping F<sub>2</sub> plants. QTL mapping for PEDS was performed, identifying one minor QTL explaining 1.93% of the phenotypic variance, one major QTL explaining 23.79% of the phenotypic variance and 50 epistatic QTL explaining 94.40% of the total phenotypic variance. The major QTL was validated in three environments. These results suggested the importance of not only major and minor QTL, but the genome-wide epistasis as well, in influencing the variation of PEDS.

## Materials And Methods

### Plant materials

An F<sub>2</sub> population was developed through a cross between waxy maize inbred line SICAU1212 and teosinte MT1 (*Z. mays* ssp. *mexicana*). SICAU1212 ear has two ranks with two spikelets per cupule (paired spikelets), whereas MT1 ear has two ranks with one spikelet per cupule (single spikelet), thus excluding the effect of number of ranks, which is essential for investigation of single vs. paired spikelets. SICAU1212 was derived from a waxy maize landrace Silunuo by 10 consecutive generations of self-pollination, and had multiple primordial traits of maize, including small stature, ramification, stooling, narrow leaves, and small spike. The history of Silunuo cultivation in Yunnan Province of China can be traced back to at least 1890 (Zeng and Pu 1981; Tian et al. 2009).

### Field trials and trait measurements

The F<sub>2</sub> population with 409 plants and their parents were grown at Ya'an city in Sichuan Province, China (11YA, 30°N, 104°02'E, altitude 580 m) in April 2011. To validate the major QTL identified in the 11YA environment, the F<sub>2</sub> population was grown in three environments as follows: 108 plants at Wenjiang district in Sichuan Province, China (17WJ, 30°40'N, 104°04'E, altitude 750 m) in April 2017, 115 plants at Ya'an city in Sichuan Province, China (17YA, 30°N, 103°02'E, altitude 580 m) in April 2017, and 96 plants at Jinghong city in Yunnan Province, China (17JH, 22°01'N, 100°58'E, altitude 551 m) in August 2017.

PEDS was defined by Doebley et al. (1990) as the percentage of cupules lacking the pediculate spikelet, and we followed it. In order to easily and accurately investigate the phenotype, the single and paired spikelets on the basal-most secondary lateral inflorescence were counted as soon as the silks of plant were visible, avoiding the interference of floral organs with developmental failure. The phenotypic data were cubic root transformed to reduce skewness and kurtosis (Doebley et al. 1990). Basic statistical analysis of PEDS was performed using SPSS19.0 software (<http://www.spss.com>).

### Genotyping and construction of genetic linkage map

Genomic DNA was extracted from young leaves of F<sub>2</sub> population (162 randomly selected plants from the 409-plant population, and 43 plants with PEDS >20% selected from the rest of population), their F<sub>1</sub> and parents in the 11YA environment using the cetyl trimethyl ammonium bromide (CTAB) method (Saghai-Marouf et al. 1984). The oligonucleotides pool assay (OPA) consisting of 3072 well-distributed and high-quality SNPs was used to genotype the plants mentioned above using the procedure described by Fan et al (2006) and Hou et al (2015). The SNP markers showed polymorphism between the parents SICAU1212 and MT1 and heterozygotes allele in the 162 F<sub>2</sub> plants, excluding obviously segregation distortion markers in SICAU1212 or MT1 allele less than 21, or heterozygotes allele less than 41. The chi-square analysis was conducted for the segregation ratio of the remaining SNP markers. The genetic linkage map was constructed by using MAPMAKER/EXP 3.0 with LOD threshold >3.0, and the genetic distances were calculated by the Kosambi mapping function (Lander et al. 1987; Kosambi 2016).

## QTL analysis

QTL for PEDS were identified using two approaches: one was QTL mapping in 162 randomly selected plants, and another was selective genotyping in 86 plants with extreme traits. Combined with the SNP-based linkage genetic map and phenotyping of 162 F<sub>2</sub> plants, the main QTL and epistatic effects for PEDS were identified in the QTL IciMapping 4.1 (<http://www.isbreeding.net/>) using the inclusive composite interval mapping of additive (ICIM-ADD) and two epistatic QTL (ICIM-EPI) modules (Li et al. 2015). Before QTL mapping, the missing phenotypes were removed; to detect additive QTL, walking speed was set at 1.0 cM, probability in the stepwise regression was 0.001, and threshold LOD scores were calculated using 1000 permutations with a type 1 error of 0.05. Epistatic QTL were identified by using walking speed of 5 cM, probability of 0.0001 in stepwise regression, and threshold LOD of 5.0.

A subset of 86 plants, namely 43 plants with PEDS = 0 chosen from the 162 F<sub>2</sub> plants and 43 plants with PEDS >20% selected from 409 plants representing the most extreme phenotypes, were used for selective genotyping to identify QTL for PEDS. The detection of QTL for PEDS was performed in the QTL IciMapping 4.1 using the selective genotyping mapping (SGM) and the inclusive composite interval mapping of additive (ICIM-ADD) modules. The parameters of threshold LOD were the same as that for additive QTL detection described above. QTL designations followed the standard nomenclature of McCouch et al. (1997). For example, for QTL *qPEDS3.1*, q represents a QTL, peds is the abbreviation for "Percentage of cupules lacking the pedicellate spikelet", 3 indicates that the QTL was located on the chromosome 3, and 1 is the serial number of that QTL; the QTL had the same serial number when mapped within the same marker interval or when sharing a common marker.

## Validation of QTL regions

The two QTL on chromosomes 1L and 3S identified in the 11YA environment were validated in three environments. First, a total of 36 InDel markers (Liu et al. 2015) located in the QTL regions were chosen to detect polymorphism between SICAU1212 and MT1. The procedure of PCR amplification was conducted as described in our previous reports (CHEN et al. 2017), and denatured amplified products were separated on 6% polyacrylamide gels and visualized by silver staining (Sanguinetti et al. 1994). Then, the co-dominant and non-segregation distortion markers were utilized to construct the local genetic linkage map. With the phenotypic data, QTL mapping was performed following the procedures described above.

## Results

### Phenotypic variation in the trait

The ear of maize SICAU1212 is about 12 cm, presenting two ranks with 21–24 cupules per rank and two spikelets per cupule (paired spikelets); in contrast, the ear of teosinte MT1 is about 7 cm, presenting two ranks with six cupules per rank and one spikelet per cupule (Fig. 1). The differences in PEDS between SICAU1212 and MT1 were distinct, namely PEDS = 0% in SICAU1212 versus PEDS = 100% in MT1; the PEDS of F<sub>1</sub> plants equaled 0%, indicating that maize was completely dominant to teosinte (Table 1). The mean values of PEDS were 7.63%, 8.77%, 8.32%, and 13.35% in the F<sub>2</sub> populations, with a range from 0–100%, in the four environments, respectively. The ratio of individuals with PEDS > 0% in the F<sub>2</sub> population ranged from 1/6 (17WJ) to 1/4 (17JH). In addition, at least one plant with all single spikelets (PEDS = 100%) was observed in each environment. However, the phenotypes of F<sub>2</sub> populations did not follow a normal distribution, which was instead significantly skewed toward the maize phenotype (Fig. 2).

Table 1  
Phenotypic performance of the PEDS in parents and F<sub>2</sub> populations under four environments

| Env. <sup>a</sup>   | population size | MT1 (%) <sup>b</sup> | SICAU1212 (%) <sup>c</sup> | Mean (%) <sup>d</sup> | Min (%) | Max (%) | Skewness | Kurtosis |
|---|-----------------|----------------------|----------------------------|-----------------------|---------|---------|----------|----------|
| 11YA  | 163             | 100                  | 0                          | 7.63                  | 0       | 100     | 3.13     | 9.16     |
| 17WJ  | 108             | 100                  | 0                          | 8.77                  | 0       | 100     | 2.83     | 7.14     |
| 17YA  | 115             | 100                  | 0                          | 8.32                  | 0       | 100     | 2.96     | 8.45     |
| 17JH  | 96              | 100                  | 0                          | 13.35                 | 0       | 100     | 2.15     | 3.31     |
| <sup>a</sup> Env. represents environments. 11YA, 17WJ, 17YA and 17JH represent Ya'an 2011, Wenjiang 2017, Ya'an 2017 and Jinghong 2017, respectively. |                 |                      |                            |                       |         |         |          |          |
| <sup>b</sup> All cupules of the ear are single spikelet.  |                 |                      |                            |                       |         |         |          |          |
| <sup>c</sup> All cupules of the ear are paired spikelet.  |                 |                      |                            |                       |         |         |          |          |

## Linkage Map And Segregation Distortion Regions

The oligonucleotides pool assay (OPA) with 3072 SNPs was utilized to genotype the F<sub>2</sub> plants, including 162 randomly selected plants, 43 plants with PEDS > 20%, the parents, and F<sub>1</sub>. As a result, 839 SNPs showed polymorphisms between the parents and heterozygote in the F<sub>1</sub>, with the ratio of 27.31%. To exclude the obviously segregation distortion markers, 586 SNPs were used to construct the genetic linkage map. The linkage map covered all 10 maize chromosomes, and the number of markers ranged from 36 on chromosome 10 to 81 on chromosome 8. It spanned a total length of 1758.29 cM, with an average marker interval of 3.00 cM; the biggest linkage distance between two adjacent markers was 32.12 cM, located on chromosome 3 between PZE-103118170 and PZE-103150482 (Fig. 3).

Fifteen strong segregation distortion regions (SDRs), including 220 of 586 SNPs (37.54%), were detected in the linkage map, and were located on chromosomes 1 to 9 (Table 2, Fig. 3). The number of markers contained in each SDR ranged from 3 (*SDR8-2*) to 32 (*SDR8-1*). The markers in *SDR1-1*, *SDR5-1*, *SDR8-1*, and *SDR9-1* were skewed towards SICAU1212, whereas those in *SDR2-1*, *SDR3-1*, *SDR3-2*, *SDR6-1*, *SDR6-2*, *SDR6-3*, *SDR6-4*, *SDR8-2*, and *SDR8-3* deviated towards heterozygote. Additionally, *SDR4-1* deviated towards SICAU1212 and heterozygote, whereas *SDR7-1* deviated towards MT1 and heterozygote.

Table 2  
Summary of segregation distortion regions (SDRs) in the F<sub>2</sub> population of SICAU1212 × MT1

| SDRs          | Chromosome | marker interval                 | Physical position (Mb) <sup>a</sup> | Number of SICAU1212 allele <sup>b</sup> | Number of MT1 allele <sup>b</sup> | Number of heterozygotes allele <sup>b</sup> | Direction of skewness   |
|---------------|------------|---------------------------------|-------------------------------------|---|-----------------------------------|---|-------------------------|
| <i>SDR1-1</i> | 1          | SYN35048-PZE-101229195          | 192.40-278.91                       | 56.8                                    | 21.3                              | 84.1  | SICAU1212               |
| <i>SDR2-1</i> | 2          | PZE-102060224-PZE-102142740     | 38.54-189.63                        | 43.9                                    | 22.1                              | 96.2  | Heterozygote            |
| <i>SDR3-1</i> | 3          | PZE-103032109-PZE-103035540     | 24.73-28.84                         | 43.5                                    | 23.5                              | 94  | Heterozygote            |
| <i>SDR3-2</i> | 3          | PZE-103059411-SYN30483          | 113.07-225.91                       | 44.4                                    | 24.4                              | 93.4  | Heterozygote            |
| <i>SDR4-1</i> | 4          | PZE-104005482-PZE-104072386     | 1.45-143.51                         | 49.7                                    | 21.2                              | 90.7  | SICAU1212, Heterozygote |
| <i>SDR5-1</i> | 5          | PZE-105150391-SYN36222          | 201.87-212.37                       | 54.1                                    | 23.4                              | 84.6  | SICAU1212               |
| <i>SDR6-1</i> | 6          | PZE-106033961-PZE-106035361     | 79.46-82.61                         | 45.5                                    | 22.5                              | 94.3  | Heterozygote            |
| <i>SDR6-2</i> | 6          | PZE-106058198-SYN38352          | 107.09-123.61                       | 40.7                                    | 23.1                              | 98  | Heterozygote            |
| <i>SDR6-3</i> | 6          | PZE-106078419-PZE-106081334     | 133.83-138.43                       | 39.2                                    | 23.7                              | 99.5  | Heterozygote            |
| <i>SDR6-4</i> | 6          | SYN4807-PZE-106104150           | 150.68-155.45                       | 33.8                                    | 27.1                              | 101.8                                       | Heterozygote            |
| <i>SDR7-1</i> | 7          | SYN36193-PUT-163a-71300000-3068 | 1.97-133.94                         | 23.2                                    | 49.7                              | 89.2  | MT1, Heterozygote       |
| <i>SDR8-1</i> | 8          | SYN3483-PZE-108052803           | 22.68-93.92                         | 56.9                                    | 28.2                              | 76.5  | SICAU1212               |
| <i>SDR8-2</i> | 8          | PZE-108056925-PZE-108060445     | 101.96-108.03                       | 45.6                                    | 22.7                              | 94.3  | Heterozygote            |
| <i>SDR8-3</i> | 8          | PZE-108096732-PZE-108110152     | 152.76-162.99                       | 26.6                                    | 40.4                              | 95.8  | Heterozygote            |
| <i>SDR9-1</i> | 9          | PZE-109038289-PZE-109047418     | 56.59-62.25                         | 53.5                                    | 38.8                              | 68.8  | SICAU1212               |

<sup>a</sup> Physical position is based on B73 RefGen V2 sequence.

<sup>b</sup> The number is the average value.

## Main And Epistatic Effect Qtl For Peds

Taking advantage of the SNP-based linkage map and precise phenotypic data, two QTL for PEDS were identified (Table 3, Fig. 3) in the F<sub>2</sub> population with 162 randomly selected plants in the 11YA environment; they were located on chromosomes 1L (*qPEDS1.1*) and 3S (*qPEDS3.1*), were flanked by markers PZE-101196838-PUT-163a-76013247-3735 and PZE-103018221-SYN28119, and accounted for 1.93% and 23.79% of the phenotypic variance, respectively. In addition, the SNP marker with the highest LOD score of 4.485 was located at SYN28119, as identified using the SGM module in the population of 86 extreme phenotypes. Moreover, the major QTL (*qPEDS3.1*) was also detected in the same population using the ICIM-ADD module; it was located between markers PZE-103018221 and SYN28119, and explained 64.01% of the phenotypic variance. The additive effect of all QTL was negative, indicating that the alleles that increased the expression of PEDS were from MT1. The *qPEDS1.1* showed the additive effect due to the dominance/additive ratio being 0.17, whereas *qPEDS3.1* showed the dominance effect due to the dominance/additive ratio being 0.84 (Edwards et al. 1987).

Table 3  
Summary of significant QTL for PEDS identified in the F<sub>2</sub> populations under four environments

| ENV. <sup>a</sup>   | QTL <sup>b</sup>            | Chromosome | Marker interval                      | Physical position (Mb) <sup>c</sup> | position (cM) | Range (cM)  | LOD <sup>d</sup> | PVE (%) <sup>e</sup> | Add <sup>f</sup> | Dom   | Gene action <sup>g</sup> |
|---|-----------------------------|------------|--------------------------------------|-------------------------------------|---------------|-------------|------------------|----------------------|------------------|-------|--------------------------|
| 11YA  | <i>qPEDS1.1<sup>R</sup></i> | 1          | PZE-101196838-PUT-163a-76013247-3735 | 245.03-270.98                       | 229           | 221.5-229.5 | 1.31             | 1.93                 | -0.06            | -0.01 | A                        |
|   | <i>qPEDS3.1<sup>R</sup></i> | 3          | PZE-103018221-SYN28119               | 10.52-20.40                         | 43            | 41.5-44.5   | 49.95            | 23.79                | -0.37            | -0.38 | D                        |
|   | <i>qPEDS3.1<sup>S</sup></i> | 3          | PZE-103018221-SYN28119               | 10.52-20.40                         | 43            | 40.5-45.5   | 29.41            | 64.01                | -0.39            | -0.33 | D                        |
| 17WJ  | <i>qPEDS3.1</i>             | 3          | chr3-14843386-chr3-19759100          | 14.84-19.75                         | 24            | 22.5-26.5   | 9.60             | 31.05                | -0.25            | -0.24 | D                        |
| 17YA  | <i>qPEDS3.1</i>             | 3          | chr3-14843386-chr3-19759100          | 14.84-19.75                         | 31            | 27.5-33.5   | 12.53            | 38.94                | -0.23            | -0.19 | D                        |
| 17JH  | <i>qPEDS3.1</i>             | 3          | chr3-11534053-chr3-14843386          | 11.53-14.84                         | 16            | 15.5-19.5   | 4.68             | 23.16                | -0.15            | -0.27 | OD                       |
| <sup>a</sup> Env. represents environments. 11YA, 17WJ, 17YA and 17JH represent Ya'an 2011, Wenjiang 2017, Ya'an 2017 and Jinghong 2017, respectively  |                             |            |                                      |                                     |               |             |                  |                      |                  |       |                          |
| <sup>b</sup> The QTL with R in the upper right corner indicates that this QTL was detected in the F <sub>2</sub> population with 162 random plants; while the QTL with S in the upper right corner indicates that this QTL was detected in the 86 extreme phenotype plants. |                             |            |                                      |                                     |               |             |                  |                      |                  |       |                          |
| <sup>c</sup> LOD score  |                             |            |                                      |                                     |               |             |                  |                      |                  |       |                          |
| <sup>d</sup> Physical position is based on B73 RefGen V2 sequence.  |                             |            |                                      |                                     |               |             |                  |                      |                  |       |                          |
| <sup>e</sup> Phenotypic variation explained by each QTL.  |                             |            |                                      |                                     |               |             |                  |                      |                  |       |                          |
| <sup>f</sup> A negative additive effect indicates that the MT1 allele increased the phenotypic value.   |                             |            |                                      |                                     |               |             |                  |                      |                  |       |                          |
| <sup>g</sup> A, D, and OD represent additive, dominance, and over-dominance effect, respectively, based on EDWARDS et al. (1987)  |                             |            |                                      |                                     |               |             |                  |                      |                  |       |                          |

In addition to the main effect QTL for PEDS, 50 pairs of epistatic QTL (named as *EPqpedS-1* to *EPqpedS-50*) were identified, being distributed on all 10 chromosomes (Table 4, Fig. 4). The total phenotypic variance explained (PVE) by these 50 epistatic QTL was 94.40%, and the phenotypic variance explained by each epistatic QTL ranged from 0.68–4.12%. However, these 50 epistatic QTL were divided into two categories based on the additive-by-additive effect (Li et al. 2015). One category, including 29 epistatic QTL, had a negative effect that decreased the expression of PEDS, and accounted for 55.59% of the phenotypic variance; another category consisting of 21 epistatic QTL had a positive effect that increased the expression of PEDS, and accounted for 38.81% of the phenotypic variance. Among them, five epistatic QTL (*EPqpedS-6* to *EPqpedS-10*) were located in the region of *qPEDS1.1*, and nine epistatic QTL (*EPqpedS-1*, *EPqpedS-13*, and *EPqpedS-17* to *EPqpedS-23*) were in the region of *qPEDS3.1*.

Table 4  
Epistatic QTL for PEDS identified in the F<sub>2</sub> population in the 11YA environment

| Epistatic QTL     | Chromosome1 | Position (cM) <sup>1a</sup> | Marker interval1                     | Chromosome2 | Position(cM) <sup>2a</sup> | Marker intervala2           | LOD <sup>b</sup> | PVE (%) <sup>c</sup> | AA <sup>d</sup> |
|-------------------|-------------|-----------------------------|--------------------------------------|-------------|----------------------------|-----------------------------|------------------|----------------------|-----------------|
| <i>EPqped</i> -1  | 1           | 15                          | PZE-101003135-SYN14143               | 3           | 40                         | PZE-103018221-SYN28119      | 46.52            | 4.12                 | -0.019          |
| <i>EPqped</i> -2  | 1           | 20                          | PZE-101003135-SYN14143               | 5           | 55                         | PZE-105054212-PZE-105056823 | 8.49             | 1.71                 | 0.167           |
| <i>EPqped</i> -3  | 1           | 25                          | PZE-101003135-SYN14143               | 4           | 30                         | PZE-104026267-PZE-104031374 | 11.72            | 1.07                 | -0.349          |
| <i>EPqped</i> -4  | 1           | 25                          | PZE-101003135-SYN14143               | 8           | 175                        | PZE-108118826-PZE-108131283 | 7.35             | 0.99                 | -0.190          |
| <i>EPqped</i> -5  | 1           | 195                         | PZE-101150513-PZE-101161892          | 9           | 110                        | PZE-109086476-PZE-109094249 | 8.79             | 1.75                 | 0.001           |
| <i>EPqped</i> -6  | 1           | 225                         | PZE-101196838-PUT-163a-76013247-3735 | 2           | 35                         | SYN7604-PZE-102040682       | 8.61             | 1.24                 | -0.193          |
| <i>EPqped</i> -7  | 1           | 225                         | PZE-101196838-PUT-163a-76013247-3735 | 6           | 15                         | PZE-106005121-PZE-106020499 | 7.14             | 1.08                 | -0.323          |
| <i>EPqped</i> -8  | 1           | 225                         | PZE-101196838-PUT-163a-76013247-3735 | 7           | 95                         | PZE-107052459-PZE-107074405 | 6.32             | 0.86                 | 0.123           |
| <i>EPqped</i> -9  | 1           | 225                         | PZE-101196838-PUT-163a-76013247-3735 | 10          | 40                         | PZE-110009618-PZE-110013181 | 7.98             | 0.77                 | -0.134          |
| <i>EPqped</i> -10 | 1           | 235                         | PZE-101222419-PZE-101229195          | 1           | 240                        | PZE-101222419-PZE-101229195 | 21.19            | 2.08                 | 0.025           |
| <i>EPqped</i> -11 | 2           | 10                          | SYN10369-PZE-102017883               | 10          | 65                         | PZE-110028531-PZE-110036318 | 5.89             | 0.70                 | 0.202           |
| <i>EPqped</i> -12 | 2           | 30                          | SYN7604-PZE-102040682                | 2           | 35                         | SYN7604-PZE-102040682       | 35.32            | 2.13                 | -0.331          |

<sup>a</sup> The numbers represent the genetic position on the genetic linkage map.

<sup>b</sup> LOD score.

<sup>c</sup> Phenotypic variation explained by epistatic QTL.

<sup>d</sup> Additive by additive effect of QTL at the two scanning positions. The positive and negative values indicate the epistatic QTL increased and decreased the phenotypic value, respectively.

| Epistatic QTL     | Chromosome1 | Position (cM) <sup>1a</sup> | Marker interval1            | Chromosome2 | Position(cM) <sup>2a</sup> | Marker interval2            | LOD <sup>b</sup> | PVE (%) <sup>c</sup> | AA <sup>d</sup> |
|-------------------|-------------|-----------------------------|-----------------------------|-------------|----------------------------|-----------------------------|------------------|----------------------|-----------------|
| <i>EPqpeds-13</i> | 2           | 35                          | SYN7604-PZE-102040682       | 3           | 40                         | PZE-103018221-SYN28119      | 46.38            | 4.10                 | 0.067           |
| <i>EPqpeds-14</i> | 2           | 35                          | SYN7604-PZE-102040682       | 4           | 30                         | PZE-104026267-PZE-104031374 | 8.95             | 0.90                 | 0.051           |
| <i>EPqpeds-15</i> | 2           | 40                          | SYN7604-PZE-102040682       | 9           | 110                        | PZE-109086476-PZE-109094249 | 7.68             | 0.91                 | -0.239          |
| <i>EPqpeds-16</i> | 2           | 120                         | PZE-102137972-PZE-102139681 | 7           | 30                         | SYN4762-SYN20419            | 5.28             | 0.68                 | 0.214           |
| <i>EPqpeds-17</i> | 3           | 40                          | PZE-103018221-SYN28119      | 3           | 70                         | PZE-103037508-PZE-103040868 | 50.31            | 4.12                 | -0.006          |
| <i>EPqpeds-18</i> | 3           | 40                          | PZE-103018221-SYN28119      | 4           | 80                         | SYN26775-PZE-104088242      | 25.66            | 4.10                 | 0.153           |
| <i>EPqpeds-19</i> | 3           | 40                          | PZE-103018221-SYN28119      | 5           | 110                        | SYN34468-SYN7361            | 38.02            | 4.10                 | -0.138          |
| <i>EPqpeds-20</i> | 3           | 40                          | PZE-103018221-SYN28119      | 6           | 20                         | PZE-106005121-PZE-106020499 | 43.81            | 4.10                 | -0.087          |
| <i>EPqpeds-21</i> | 3           | 40                          | PZE-103018221-SYN28119      | 8           | 15                         | PZA00058.6-PZE-108016906    | 42.17            | 4.10                 | -0.082          |
| <i>EPqpeds-22</i> | 3           | 40                          | PZE-103018221-SYN28119      | 9           | 85                         | PZE-109066773-PZE-109086476 | 52.46            | 4.10                 | -0.253          |
| <i>EPqpeds-23</i> | 3           | 40                          | PZE-103018221-SYN28119      | 10          | 10                         | SYN17609-SYN17100           | 42.71            | 4.10                 | 0.149           |
| <i>EPqpeds-24</i> | 3           | 50                          | SYN28119-PZE-103032109      | 7           | 100                        | PZE-107052459-PZE-107074405 | 48.65            | 4.10                 | 0.067           |
| <i>EPqpeds-25</i> | 4           | 10                          | SYN5712-PZE-104021281       | 5           | 80                         | PZE-105061606-PZA02676.2    | 7.23             | 1.00                 | -0.308          |
| <i>EPqpeds-26</i> | 4           | 25                          | PZE-104024382-PZE-104024520 | 10          | 30                         | SYN17100-PZE-110009618      | 11.84            | 0.95                 | -0.181          |

<sup>a</sup> The numbers represent the genetic position on the genetic linkage map.

<sup>b</sup> LOD score.

<sup>c</sup> Phenotypic variation explained by epistatic QTL.

<sup>d</sup> Additive by additive effect of QTL at the two scanning positions. The positive and negative values indicate the epistatic QTL increased and decreased the phenotypic value, respectively.

| Epistatic QTL     | Chromosome1 | Position (cM) <sup>1a</sup> | Marker interval1                     | Chromosome2 | Position(cM) <sup>2a</sup> | Marker interval2            | LOD <sup>b</sup> | PVE (%) <sup>c</sup> | AA <sup>d</sup> |
|-------------------|-------------|-----------------------------|--------------------------------------|-------------|----------------------------|-----------------------------|------------------|----------------------|-----------------|
| <i>EPqpeds-27</i> | 4           | 30                          | PZE-104026267-PZE-104031374          | 4           | 35                         | PZE-104031374-PZE-104039411 | 12.22            | 1.39                 | 0.030           |
| <i>EPqpeds-28</i> | 4           | 30                          | PZE-104026267-PZE-104031374          | 6           | 5                          | PZE-106005121-PZE-106020499 | 8.99             | 1.62                 | 0.270           |
| <i>EPqpeds-29</i> | 4           | 30                          | PZE-104026267-PZE-104031374          | 7           | 190                        | PZE-107130789-PZE-107137037 | 10.51            | 0.90                 | -0.285          |
| <i>EPqpeds-30</i> | 4           | 30                          | PZE-104026267-PZE-104031374          | 8           | 155                        | PZE-108112111-PZE-108118826 | 12.00            | 0.85                 | -0.243          |
| <i>EPqpeds-31</i> | 4           | 30                          | PZE-104026267-PZE-104031374          | 9           | 85                         | PZE-109066773-PZE-109086476 | 8.36             | 0.84                 | -0.035          |
| <i>EPqpeds-32</i> | 5           | 65                          | PZE-105056823-PUT-163a-16920212-1046 | 7           | 190                        | PZE-107130789-PZE-107137037 | 14.23            | 1.75                 | 0.361           |
| <i>EPqpeds-33</i> | 5           | 75                          | ZM013514-0174-PZE-105061606          | 10          | 15                         | SYN17609-SYN17100           | 9.85             | 1.30                 | 0.040           |
| <i>EPqpeds-34</i> | 5           | 100                         | PZE-105103973-SYN35495               | 5           | 110                        | SYN34468-SYN7361            | 29.71            | 2.08                 | -0.110          |
| <i>EPqpeds-35</i> | 5           | 160                         | PZE-105163590-PZE-105167452          | 9           | 110                        | PZE-109086476-PZE-109094249 | 5.70             | 0.84                 | 0.241           |
| <i>EPqpeds-36</i> | 5           | 165                         | PZE-105167452-PZE-105171774          | 8           | 25                         | PZA00058.6-PZE-108016906    | 12.51            | 1.37                 | -0.156          |
| <i>EPqpeds-37</i> | 6           | 5                           | PZE-106005121-PZE-106020499          | 7           | 185                        | PZE-107130789-PZE-107137037 | 5.47             | 0.80                 | 0.208           |
| <i>EPqpeds-38</i> | 6           | 15                          | PZE-106005121-PZE-106020499          | 6           | 25                         | PZE-106005121-PZE-106020499 | 44.37            | 2.13                 | -0.225          |
| <i>EPqpeds-39</i> | 6           | 80                          | PZE-106053643-PZE-106058198          | 10          | 15                         | SYN17609-SYN17100           | 8.00             | 0.82                 | -0.255          |

<sup>a</sup> The numbers represent the genetic position on the genetic linkage map.

<sup>b</sup> LOD score.

<sup>c</sup> Phenotypic variation explained by epistatic QTL.

<sup>d</sup> Additive by additive effect of QTL at the two scanning positions. The positive and negative values indicate the epistatic QTL increased and decreased the phenotypic value, respectively.

| Epistatic QTL  | Chromosome1 | Position (cM) <sup>1a</sup> | Marker interval1                | Chromosome2 | Position(cM) <sup>2a</sup> | Marker interval2                | LOD <sup>b</sup> | PVE (%) <sup>c</sup> | AA <sup>d</sup> |
|--|-------------|-----------------------------|---------------------------------|-------------|----------------------------|---------------------------------|------------------|----------------------|-----------------|
| <i>EPqpeds-40</i>  | 6           | 135                         | PZE-106083588-<br>PZE-106083873 | 8           | 25                         | PZA00058.6-<br>PZE-108016906    | 7.98             | 1.36                 | 0.130           |
| <i>EPqpeds-41</i>  | 7           | 10                          | SYN36193-<br>SYN4760            | 8           | 170                        | PZE-108118826-<br>PZE-108131283 | 16.51            | 1.81                 | -0.125          |
| <i>EPqpeds-42</i>  | 7           | 10                          | SYN36193-<br>SYN4760            | 9           | 110                        | PZE-109086476-<br>PZE-109094249 | 5.93             | 1.61                 | 0.134           |
| <i>EPqpeds-43</i>  | 7           | 15                          | SYN36193-<br>SYN4760            | 10          | 65                         | PZE-110028531-<br>PZE-110036318 | 6.68             | 0.96                 | -0.121          |
| <i>EPqpeds-44</i>  | 7           | 170                         | SYN12246-<br>PZE-107130514      | 7           | 185                        | PZE-107130789-<br>PZE-107137037 | 42.18            | 2.08                 | 0.000           |
| <i>EPqpeds-45</i>  | 8           | 20                          | PZA00058.6-<br>PZE-108016906    | 8           | 35                         | PZA00058.6-<br>PZE-108016906    | 47.70            | 2.13                 | -0.287          |
| <i>EPqpeds-46</i>  | 8           | 170                         | PZE-108118826-<br>PZE-108131283 | 9           | 85                         | PZE-109066773-<br>PZE-109086476 | 16.94            | 1.79                 | -0.004          |
| <i>EPqpeds-47</i>  | 8           | 170                         | PZE-108118826-<br>PZE-108131283 | 10          | 55                         | PZE-110017983-<br>PZE-110028531 | 7.80             | 0.93                 | -0.148          |
| <i>EPqpeds-48</i>  | 9           | 85                          | PZE-109066773-<br>PZE-109086476 | 10          | 55                         | PZE-110017983-<br>PZE-110028531 | 5.18             | 0.99                 | 0.008           |
| <i>EPqpeds-49</i>  | 9           | 110                         | PZE-109086476-<br>PZE-109094249 | 9           | 125                        | ZM013385-<br>0395-PZE-109099670 | 48.48            | 2.13                 | -0.289          |
| <i>EPqpeds-50</i>  | 10          | 10                          | SYN17609-<br>SYN17100           | 10          | 30                         | SYN17100-<br>PZE-110009618      | 35.58            | 2.08                 | 0.123           |
| <sup>a</sup> The numbers represent the genetic position on the genetic linkage map.  |             |                             |                                 |             |                            |                                 |                  |                      |                 |
| <sup>b</sup> LOD score.  |             |                             |                                 |             |                            |                                 |                  |                      |                 |
| <sup>c</sup> Phenotypic variation explained by epistatic QTL.  |             |                             |                                 |             |                            |                                 |                  |                      |                 |
| <sup>d</sup> Additive by additive effect of QTL at the two scanning positions. The positive and negative values indicate the epistatic QTL increased and decreased the phenotypic value, respectively. |             |                             |                                 |             |                            |                                 |                  |                      |                 |

## Validation Of The Main Qtl

To validate the main QTL (*qPEDS1.1* and *qPEDS3.1*), 11 polymorphic InDel markers were selected from 36 InDel markers, and were utilized to construct the local genetic linkage map in the *qPEDS1.1* and *qPEDS3.1* regions. No significant QTL was detected in the *qPEDS1.1* region, whereas one major QTL (named *qPEDS3.1*) was identified in the *qPEDS3.1* region in the three environments (Table 3, Fig. 5). The QTL was flanked by markers chr3-14843386 and chr3-19759100, accounting for 31.05% and 38.94% of phenotypic variance in the 17WJ and 17YA

environments, respectively. In addition, the QTL located between chr3-11534053 and chr3-14843386 explained 23.16% of phenotypic variance in the 17YA environment. All QTLs had a negative additive effect, indicating that alleles from MT1 contributed predominantly to the phenotype.

## Discussion

### The advantage of four-row waxy maize landrace with relatively primitive morphology in studying PEDS

In order to dissect the genetic basis of single vs. paired spikelets (PEDS), the two-ranked (four rows) maize SICAU1212 (derived from four-row waxy maize landrace Silunuo) was crossed to the two-ranked (two rows) teosinte MT1 to develop an  $F_2$  population. The SNP array was used for genotyping of the  $F_2$  plants, resulted in one major, one minor and 50 epistatic QTL for PEDS were identified. There are several advantages in using SICAU1212 as a parent in the study of the origin of paired spikelets during maize domestication. Firstly, the ears of both SICAU1212 and teosinte MT1 had two ranks, avoiding the confusion potentially arising from the segregation of ranks (rows) in a multi-ranked maize-teosinte  $F_2$  population; notably, the fact all plants of the  $F_2$  population of SICAU1212  $\times$  MT1 had only two ranks meant the only difference was paired spikelets in SICAU1212 ear versus single spikelets in MT1, making it easy to investigate the PEDS phenotype, as shown in Fig. 1. For instance, the SICAU1212 ear has an average ear length of 12 cm and approximately 80 kernels in four rows, whereas the earliest maize ear was 6 cm in length with 28 kernels in four to eight rows. According to the records, the maize landrace Silunuo (SICAU1212) has been cultivated in Yunnan Province, China, since 1890 (Zeng and Pu 1981). In addition, the cluster analysis of SICAU1212, 368 maize inbred lines and eight teosintes resulted in classifying SICAU1212 and eight teosintes into the same sub-group (Figure S1, unpublished data). In other words, SICAU1212 is a relatively primitive maize in both phenotypic and genetic relationship analyses. The other advantage was that the differences in the genetic background between SICAU1212 and teosinte was small, which reduced the effect of complex genetic background on QTL mapping; the QTL for PEDS identified in this study were probably related to the transformation of spikelets from single to paired during maize domestication rather than due to maize improvement.

### The Qtl (Qpeds3.1) Was A Novel Major Qtl

In this study, two QTL for PEDS were identified, one major stable QTL (*qPEDS3.1*) located on the short arm of chromosome 3 (3S) and one minor QTL (*qPEDS1.1*) located on the long arm of chromosome 1 (1L). In previous studies, the highest frequency of QTL for PEDS was located on chromosomes 1L and 3L (Doebley et al. 1990; Doebley and Stec 1991, 1993). The QTL *qPEDS1.1* was consistent with the QTL-1L detected by Doebley et al., but the phenotypic variance (1.93%) of *qPEDS1.1* was much lower than that of QTL-1L (~19.5%); the reason may be that the mapping populations and the method of QTL mapping were different. The QTL *qPEDS3.1* was mapped on chromosome 3S, accounting for 23.16–38.94% of the phenotypic variance in the four environments tested in this study, whereas the QTL-3L located on chromosome 3L explained 13.0–46.1% of the phenotypic variance as reported by Doebley et al (Doebley and Stec 1993; Doebley et al. 1990; Doebley and Stec 1991). Similarly, one QTL explaining 5.8% of the phenotypic variance and located on chromosome 3S was also identified by Lauter and Doebley (2002a); that QTL was approximately 4 Mb away from the QTL *qPEDS3.1* based on the physical position of markers, and further verification is needed to ascertain whether they are the same. Notably, the phenotypic variance associated with *qPEDS3.1* was 64.01% (detected using selective genotyping), which was 2-fold higher than that of QTL detected in the 162 randomly selected  $F_2$  plants. One reason may be that the selective genotyping eliminated the effect of the high segregation distortion in the maize-teosinte population. Another reason may be that the obstacles in QTL mapping arising from epistatic effects can be overcome using selective genotyping analysis (Lee et al. 2014).

#### Using the high-density linkage map detected more segregation distortion regions potentially related to maize domestication

Different types of molecular markers have been used for constructing the linkage maps in the maize  $\times$  teosinte  $F_2$  or BC populations, including RFLP, AFLP, SSR and SNP, with the number of markers ranging from 58 (RFLP) to 338 (AFLP) (Mano et al. 2005; Briggs et al. 2007; Jun-qing et al. 2011; Wang et al. 2012). Comparatively, the SNP array with 3072 SNPs was used to genotype the  $F_2$  plants in this study, and 586 SNPs were used to construct the linkage map. The total length of the linkage map was 1758.29 cM, which was shorter than that of a B73  $\times$  *parviglumis*  $F_2$  population (2210.8 cM) (Wang et al. 2012) and a B73  $\times$  *mexicana*  $F_2$  population (2002.4 cM) (Jun-qing et al. 2011), but longer than that of a B73  $\times$  *diploperennis* BC population (1357.7 cM) (Wang et al. 2012), a B64  $\times$  *huehuetenangensis*  $F_2$  population (1402.4 cM) (Mano et al. 2005) and a W22  $\times$  *parviglumis*  $F_2$  population (1474.9 cM) (Briggs et al. 2007). In addition, the SNP array was also utilized to genotype the  $F_{2,3}$  population derived from a maize  $\times$  maize (R08  $\times$  Ye478) cross, and the total length of the

linkage map was 2007.9 cM, which was longer than in this study (Hou et al. 2015). The linkage map constructed in this study was a relatively high-density linkage map, which was helpful in detection of QTL and the segregation distortion regions.

Segregation distortion is a common phenomenon generally observed in the interspecies  $F_2$  populations, and is considered to be a powerful evolutionary force (Kim et al. 2014). By integrating the results of 13 different studies (Wendel et al. 1987; Doebley and Stec 1991, 1993; Dufour et al. 2001; Lu et al. 2002; Sharopova et al. 2002; Yan et al. 2003; Mano et al. 2005; Mano and Omeri 2008; Jun-qing et al. 2011; Wang et al. 2012; Cai et al. 2014), a total of 25 segregation distortion regions (SDRs) were identified on all 10 chromosomes (Table S1). Of these, *SDR1-2*, *SDR2-1* and *SDR8-3* were detected only in the linkage map of maize × maize population, whereas *SDR6-4* and *SDR8-2* were detected only in the maize × teosinte population. Notably, 17 SDRs were identified three or more times in different studies, regarded as “SDR hotspot” regions, of which, six SDRs harbored the locations of six known gametophytic factors, including *gams1* (Bin2.04), *ga7* (Bin3.09), *ga1* (Bin4.02), *ga10* (Bin5.00-5.09), *ga2* (Bin5.04-5.05), and *ga8* (Bin9.02). In addition, four of five regions located on chromosomes 1L, 2S, 3, and 4S that affected most of the differences between maize and teosinte (Doebley 1992) overlapped with the SDR hotspot regions (*SDR1-1*, *SDR2-1*, *SDR3-2*, and *SDR4-1*) identified in this study.

## Epistasis Contributed Significantly To The Variation Of Peds

In this study, 50 epistatic QTL were identified, explaining 94.40% of the total phenotypic variance, which was much larger than that of the main effect QTL (~ 25.72%), suggesting that epistasis played an important role in the variation of PEDS as also described in previous studies (Doebley and Stec 1991; Doebley et al. 1995a). Doebley and Stec (1991) detected significant epistasis between loci *umc107* and *umc92* in the  $F_2$  population derived from maize Sin2 × teosinte Doebley643, which increased the expression of PEDS; and epistasis was confirmed in the later study (Doebley et al. 1995a). In contrast, no significant epistasis was identified in the  $F_2$  population derived from maize Nay15 × teosinte Iltis&Cochrane 81 (Doebley and Stec 1993; Doebley et al. 1995a). We also did not detect epistasis between these two loci, but the locus *umc107* was close to the marker PZE-101196838 that interacted with loci on chromosomes 2, 6, 7, and 10 (*EPqpeds-6* to *EPqpeds-9*). In addition, the QTL flanked by *bnl5.46-umc42a* and *bnl8.32-umc151* detected by Doebley et al (Doebley et al. 1990; Doebley and Stec 1991) overlapped with the epistatic QTL *EPqpeds-27* and *EPqpeds-44* based on the physical position, respectively. However, no significant epistasis was identified in these two QTL regions in previous studies. The possible reason was that epistasis was affected by the genetic background (Doebley and Stec 1991). Epistasis for PEDS was detected in the teosinte genetic background and significantly increased the expression of PEDS, whereas PEDS were invariant in the maize genetic background (Doebley et al. 1995a; Lukens and Doebley 1999).

The main force of crop domestication probably came from mutations, recombination and genetic drift. In conditions where epistasis is common; mutation may be neutral or beneficial in one genetic background but deleterious in other genetic backgrounds (De Visser et al. 2011; Lehner 2011; Breen et al. 2012). In other words, epistasis could determine a consequence of mutation effect, resulting in different phenotypes of the trait (Wagner et al. 1997; Rice 1998; Nijhout 2002; Azevedo et al. 2006). Epistasis is regarded as a byproduct of the evolutionary process (Hurst 2000). In contrast, some researchers think it can determine the outcome of many evolutionary processes (Kondrashov 1988; Azevedo et al. 2006). The single spikelet in teosinte ear was completely transformed into paired spikelets in maize ear during maize domestication. The paired spikelets are dominant to single spikelet and are invariant in modern maize after stabilizing selection by breeders, suggesting that the process of selection led to the genetic robustness of paired spikelets as the beneficial trait (Waddington 1953; Azevedo et al. 2006). In the present study, 50 epistatic QTL for PEDS were identified, suggesting that epistasis may be a remnant of evolution that played an important role in selection for the genetic robustness of paired spikelets; therefore, PEDS have had a complex evolutionary process.

## Abbreviations

DISA Tendency of ear to shatter

FM floral meristems

GLUM Hardness and angle of outer glume

InDel insertion-deletion

LIBN Number of branches in primary lateral inflorescence

NILs near isogenic lines

RANK Number of rows of cupules

SDRs segregation distortion regions

SM spikelet meristems

SPM spikelet pair meristems

OPA oligonucleotides pool assay

PEDS Percentage of cupules lacking the pedicellate spikelet

QTL quantitative trait locus (loci)

## Declarations

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### Author contributions

J. L. and T. R. Conceived and designed the experiments. Z. C., K. H., Y. Y., D. T., J. N., P. L., and L. W. conducted phenotyping measurement in the field trial and Performed the experiments. Z. C. analyzed the data. Z. C. and J. L. Wrote and revised the manuscript.

### Availability of data and materials

The data are provided in the Supplement information and the materials available on request from the authors.

### Ethics approval and consent to participate

All of the authors have read and have abided by the statement of ethical standards for manuscripts submitted to Molecular Breeding.

### Consent for publication

All of the authors approved the manuscript published in Molecular Breeding..

### Conflict of interest

The authors declare no competing interests.

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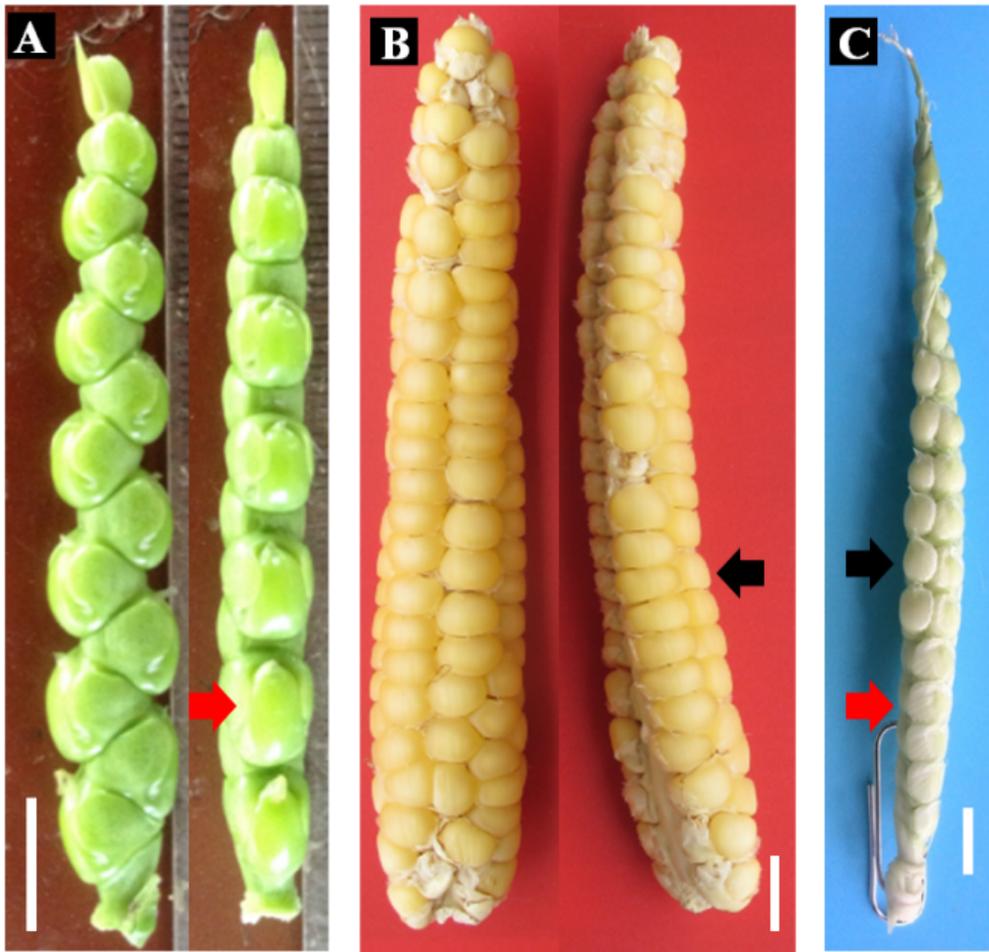
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## Figures



**Figure 1**

Ears of MT1, SICAU1212 and individual of the F2 population. (A) The teosinte (MT1) ear bears single spikelet. (B) The maize (SICAU1212) ear carries paired spikelet, and it has two ranks, that is same as that of teosinte. (C) There are both single spikelet and paired spikelet on the ear of the plant in the F2 segregating populations. The red arrows point to single spikelet and black arrows point to paired spikelet. All white bars represent 1 cm.

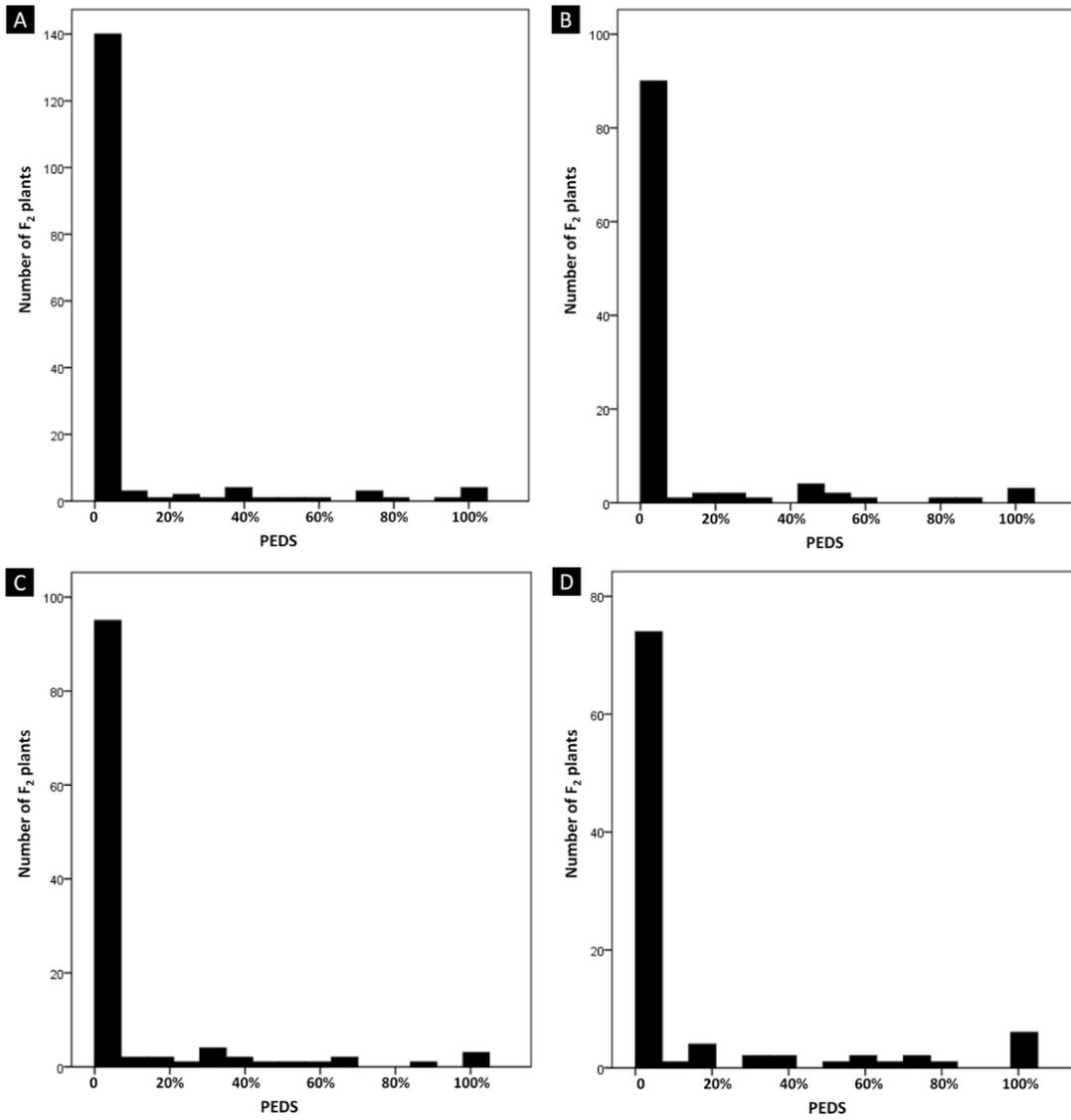


Figure 2

Frequency distribution of PEDS in the F2 populations in four environments. (A) 11YA environment, (B) 17WJ environment, (C) 17YA environment, (D) 17JH environment.

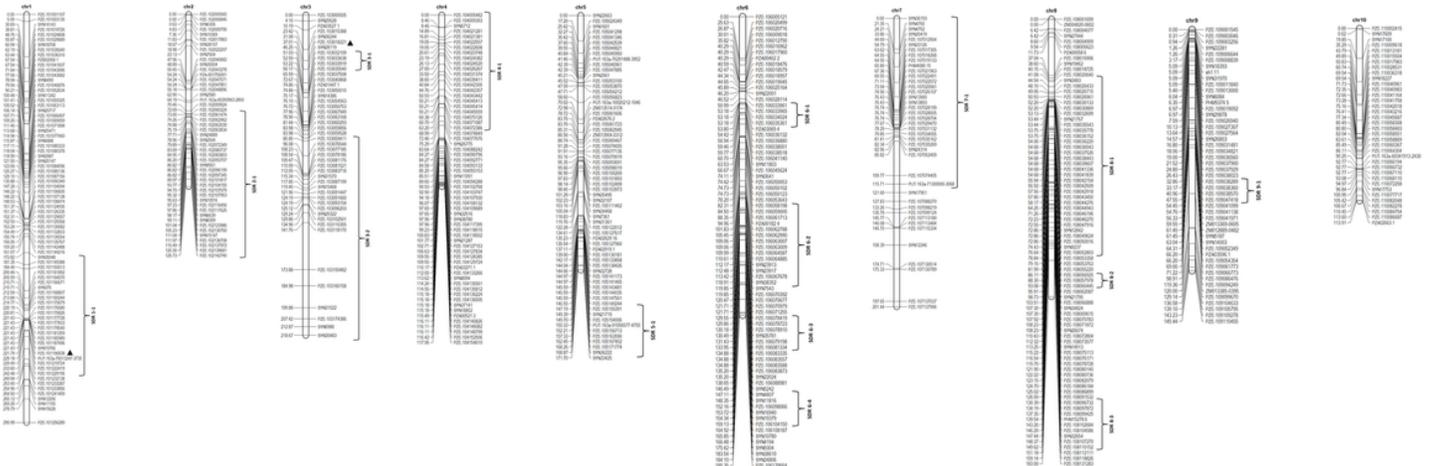
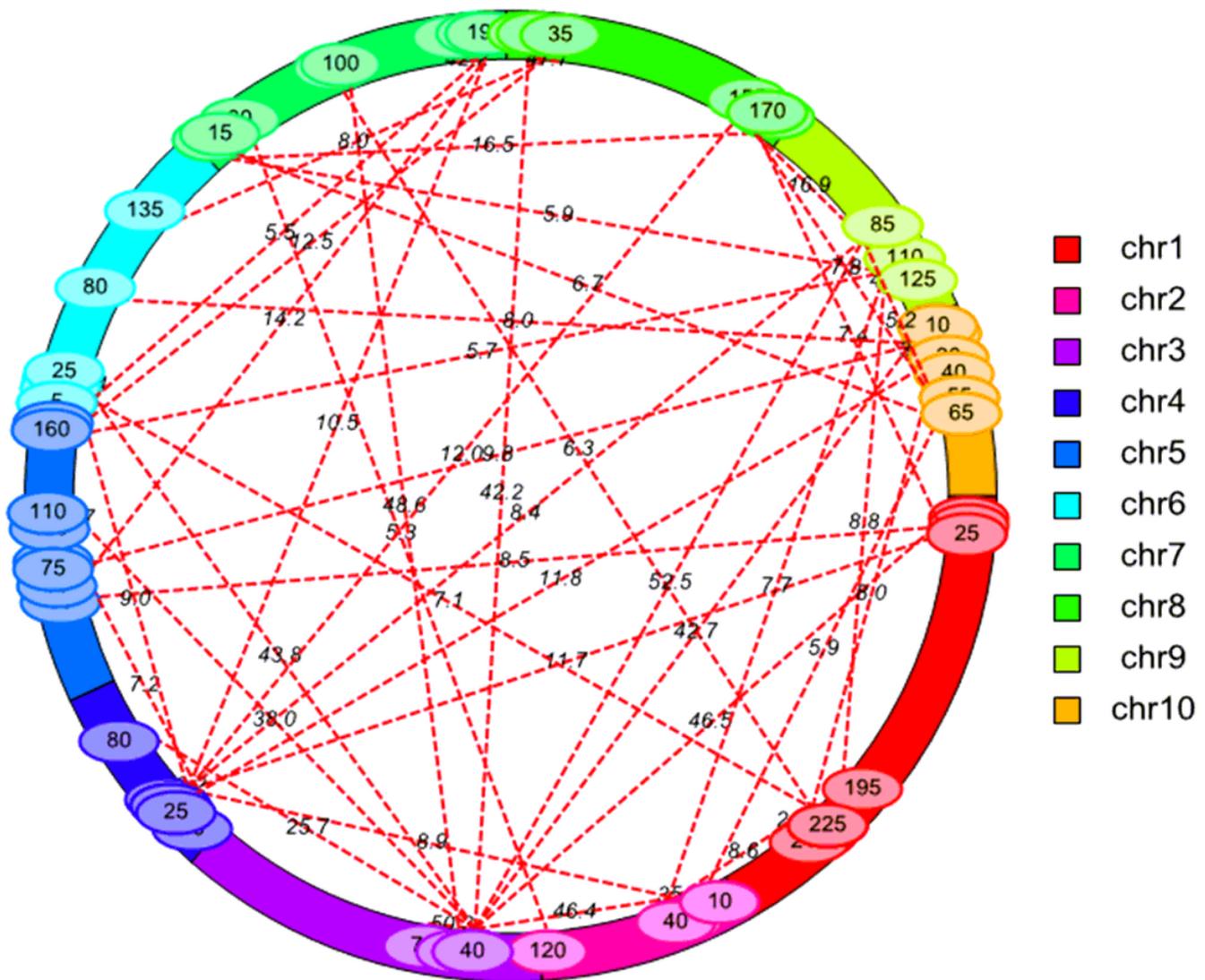


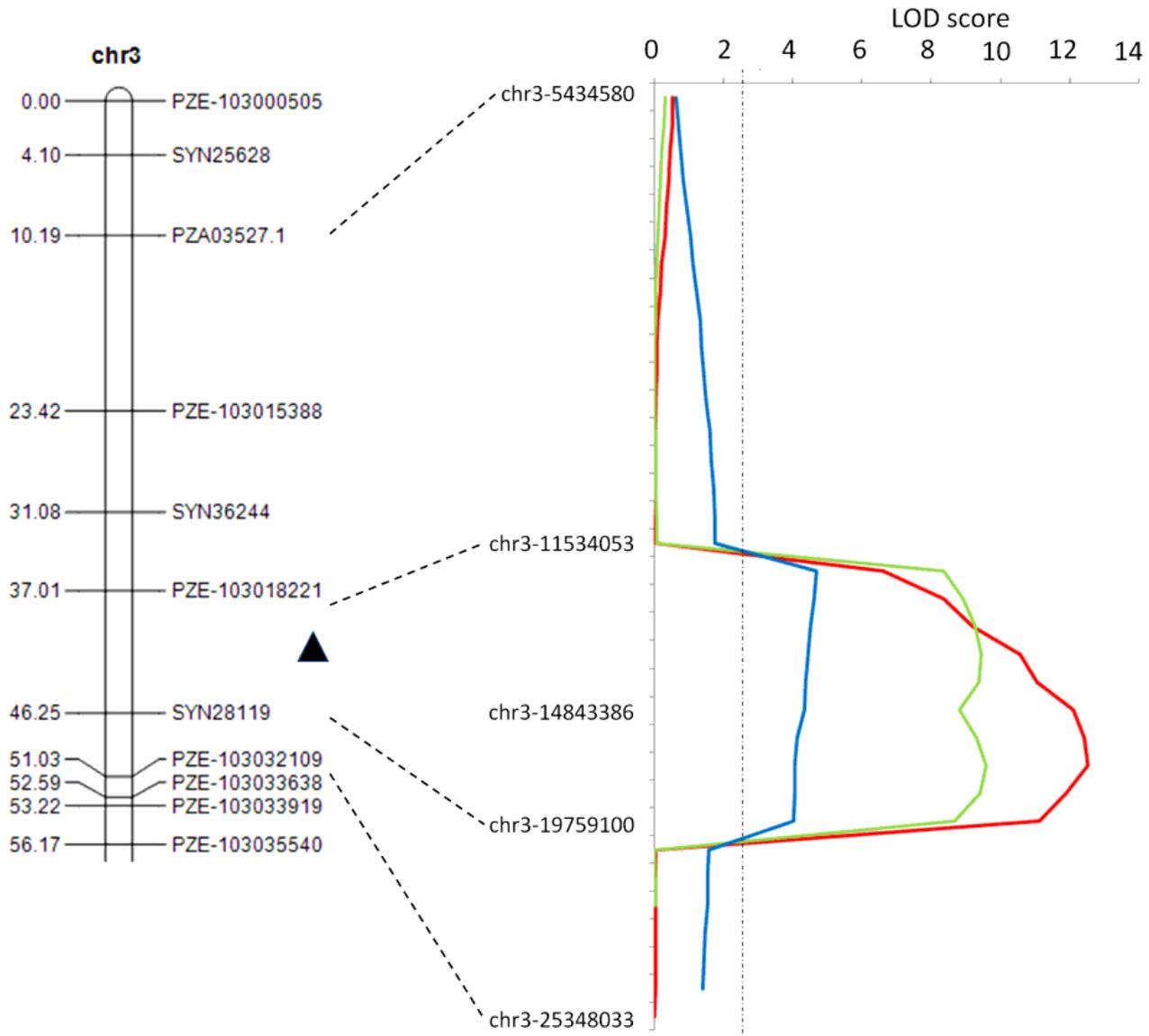
Figure 3

Molecular linkage map of the F2 population derived from SICAU1212 × MT1 in the 11YA environment. Genetic distances (cM) are shown on the left and SNP markers are indicated on the right. The triangles represent QTL for PEDS. The brackets represent segregation distortion regions (SDRs).



**Figure 4**

Genome-wide distribution of epistatic QTL for PEDS identified by ICIM-EPI in the F2 population in the 11YA environment. The linkage groups with different colors arranged in order from chromosome 1 to 10 in a colorized circle. The red long dashed lines attached to the two loci involved in epistatic interaction for PEDS, the numerical values in the colorized circle represent the genetic position on the linkage group, while the numbers on the dashed lines show the LOD values of the corresponding epistatic interactions.



**Figure 5**

Validation of the major QTL (qPDS3.1) for PEDS under three environments. The green curve, red curve and blue curve represent the LOD scores were plotted in the 17WJ, 17YA and 17JH environments, respectively. The triangle represents the major QTL for PEDS detected in the 11YA environment.