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Enhancement of tocopherols in sweet corn by marker-assisted backcrossing of *ZmVTE4*

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Abstract Vitamin E is a group of lipid-soluble antioxidants, which is indispensable and unable to be biosynthesized by the human body. Sweet corn is one of the most popular vegetables and important vitamin E resource for human. In this study, one common corn line with favorable allele on the *InDel7* (deletion) and *InDel118* (deletion) sites in the locus of *ZmVTE4* (SY999) was introduced as the donor parent line, 4 elite sweet corn lines were selected as the recipient lines. The *ZmVTE4* gene was introgressed into 4 sweet corn lines by functional molecular marker assisted selection. Two flanking SSR markers were selected to reduce the linkage drag. Few agronomic traits were investigated in the BC_1F_1 and BC_2F_1 generations and some individuals differing from the recurrent parents had been abandoned. The investigation of few agricultural traits showed no difference between the converted lines and their recipient lines. Five selected BC_3F_2 families per backcross progenies and their recurrent parent lines were sown to measure the content of tocopherols. The quantification showed that the α-tocopherol content of three backcross progenies increased except for M14. The introduction of *ZmVTE4* gene also improved the content of γ-tocopherol and total tocopherols content. The average content of γ-tocopherol and total tocopherols content were 29.11 and 36.63 μg/g in the four selected elite sweet corn lines and the average content in the four converted lines were 51.45 and 63.34 μg/g. This result indicated that *ZmVTE4* had different expression in the sh_2 genetic background or the non-target genome introgression into the recipient parent lines affecting the content of tocopherols.

Keywords Sweet corn · *ZmVTE4* · Tocopherols · Marker assisted selection

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Introduction

Sweet corn is a type of corn that is eaten fresh and depends on one or several recessive endosperm mutations that reduces the synthesis of starch and increases the accumulation of sugars or other short chain polysaccharides. It is also one of the most popular vegetables and its growing area had increased rapidly in China in recent years. Guangdong province is the important base for sweet corn producing and consumption, where 148, 500 ha were grown in 2011 (Shu-qun et al. 2013). Carbohydrates are the main components in sweet corn, while other micronutrients are of more benefit. The nutrition quality has an

important role in the consumption of sweet corn, which is related with the human health.

Vitamin E is a kind of antioxidant that quenches free radicals in cell membranes and protects polyunsaturated fatty acids from damage. It is one of the important micronutrients and is also indispensable for humans. Three kinds of tocopherols (α, γ, and δ-tocopherol) are observed in maize kernels and γ-tocopherol is the most abundant followed by α-tocopherol (Grams et al. 1970). Up to now many researches about the evaluation of tocopherols in maize had been carried out and a wide range of tocopherols concentration had been observed (Chander et al. 2008; Egesel et al. 2003; Feng et al. 2013; Rocheford et al. 2002; Wong et al. 2003).

Though the tocopherol biosynthetic pathway in maize has not been well elucidated, a few promising research results had been published. The *VTE1* gene was the first cloned gene coding tocopherol cyclase located on the chromosome 5, which was associated with the maize sucrose exported defective gene (Provencher et al. 2001; Sattler et al. 2003). The method of QTL analysis was widely applied to discover the genes and loci associated with the tocopherols in dent corn and sweet corn (Chander et al. 2008; Feng et al. 2013; Rocheford et al. 2002; Wong et al. 2003). A few main QTLs located on chromosome 5 had been detected in these researches and these regions were associated with the increasing of α-tocopherol or γ-tocopherol. Li et al. (2012) detected two insertion/deletions (InDels) within a gene (*ZmVTE4*) encoding γ-tocopherol methyltransferase, and a single nucleotide polymorphism (SNP) located on the upstream of *ZmVTE*, which were significantly associated with α-tocopherol levels in maize kernels.

It is a useful breeding strategy to introgress favorable alleles or QTLs into elite lines by marker assisted backcrossing (Blanc et al. 2008; Flint-Garcia et al. 2003; Ribaut and Hoisington 1998). The distance between the targeted gene and the flanking markers has the main influence on the efficiency of introgression and the manipulation of the linkage drag. Many empirical and simulation studies identified that the marker assisted background selection can reduce time and resources to expedite the recovery of recurrent parent genome content (Babu et al. 2005; Lee 1995; Yousef and Juvik 2002). With the development of reliable PCR-based allele specific markers such as SSRs, and SNPs, marker-assisted selection is becoming an attractive option for simply inherited traits (Babu et al. 2004).

Unlike common corn varieties, sweet corn is picked at the immature stage of kernel development and is eaten by humans as a vegetable. Enhancement of vitamin E in sweet corn is beneficial to human health. Thus, the objectives of this research were to transfer the *ZmVTE4* into 4 elite sweet corn inbred lines by marker-assisted backcrossing and evaluate the effects of this gene on tocopherol level in different sh_2 genetic backgrounds.

Materials and methods

Plant materials

In a marker-assisted backcrossing program, SY999, the high tocopherols common maize line, was used as a donor parent provided by National Maize Improvement Center of China, China Agricultural University. Four elite sh_2 sweet corn inbred lines (M01, M14, K140 and K185) bred by the South China Agricultural University were selected as the recipient parents and the polymorphisms were screened by the two specific functional marker, *InDel7* and *InDel118* (Li et al. 2012). SY999 has the favorable alleles on the *InDel7* (deletion) and *InDel118* (deletion) sites with α-tocopherol content of 1.77 μg g^{-1} and total tocopherol content 65.41 μg g^{-1} on a dry weight basis. The four elite sweet corn lines had α-tocopherol content ranging from 3.14 to 9.26 μg g^{-1} and total tocopherol content ranging from 16.56 to 57.43 μg g^{-1} on a dry weight basis. The α-tocopherol content in sweet lines was higher than that of the donor parent because of less starch in endosperm.

Target gene and marker assay

Two functional markers, *InDel7* and *InDel118* were selected as the foreground markers based on Li et al. (2012). Two SSR markers, *bnlg1237* and *phi085* were selected as flanking markers based on the linkage map of Chander et al. (2008) to reduce the linkage drag. The distance between *bnlg1237* and *ZmVTE4* and the distance between *phi085* and *ZmVTE4* were 10.1 and 11.9 cM, respectively. Both two flanking markers and two functional markers (*InDel7* and *InDel118*) were

used in initial polymorphism analysis with SY999 and four sweet corn inbred lines, for checking their feasibility to be used as foreground selection marker for the *ZmVTE4* gene. A total of 225 SSR markers spanning all the bin locations in a maize SSR consensus map (http://www.maizegdb.org) were selected for background selection. Of the 225 markers, the numbers of polymorphic markers between SY999 and the 4 sweet corn lines were 71, 73, 73, 74 and 70, respectively. The different bands in these markers were distinctive and stable locating evenly on the whole genome. Young and healthy leaf tissue was collected and ground into fine powder in liquid nitrogen to extract the genomic DNA according to the CTAB method (Doyle and Doyle 1987). The genomic DNA was diluted to a concentration of 25 ng/L with 1*TE buffer containing 10 mg/ml of RNase A and stored at −20 °C before running the polymerase chain reaction (PCR).

Selection procedure

In order to exclude the effect of cytoplasmic inheritance, the crosses were made using the four sweet corn lines as female and the donor line SY999 as the pollen parent. Five ears per backcross from the cross of F_1 and their parent lines were harvested in the spring of 2011. In the autumn of 2011, about 200 BC_1F_1 shrunk seeds (sh_2sh_2) were sown and the selection in BC_1F_1 individuals was carried out. The individuals with heterozygotes for the *ZmVTE4* gene functional marker were selected to cross with recipient lines. Out of the selected individuals, we identified individuals that were single or double recombinants for either or both of the flanking markers. Seven ears were harvested from each selected BC_1F_1 plants, of which three to five BC_2F_1 ears were single recombinants with the closest flanking marker, bnlg1237.

In the spring of 2012, about 150 BC_2F_1 seeds with *ZmVTE4* from the three to five selected single recombinants were sown and selection in these individuals was carried out according to the criteria that they were heterozygous at the functional marker locus and homozygous for recurrent parent alleles at the two flanking loci. The backcross was performed between these selected individuals and the recurrent parent lines and the BC_3F_1 ears were obtained.

In the autumn of 2012, about 150 BC_3F_1 seeds with heterozygous *ZmVTE4* from each backcross were sown. These individuals with heterozygous site at the functional marker and homozygous sites for recurrent parent alleles at the two flanking loci were selected and self-pollinated. At this stage, whole genome selection employing polymorphic SSR markers was done on these individuals heterozygous at the *ZmVTE4* locus to identify individuals with the highest amount of recurrent parent genome. These individuals with heterozygous at the *ZmVTE4* locus and higher amount of recurrent parent genome were self-pollinated to get the ears.

These BC_3F_2 families were sown at two rows per ear and each plant per families was extracted DNA to identify the genotype of the functional marker. These homozygous families and their responding parent lines were self-pollinated and harvest to quantify the tocopherols content. Five families with higher total tocopherols content from each backcross progenies had been selected as the converted sweet corn lines. A total of 20 selected families and their parents were sown in the autumn of 2013 in a complete randomized block design with 2 replications to quantify the tocopherol components content. Every family was sown by 2 rows with 10 plants per row in a replication. We had investigated four agronomy traits such as plant height, ear height, No. of tassel branch and stalk diameter. The plant height is the height from the ground to the top and the ear height is the height from the ground to the first ear. The stalk diameter is the diameter of the third stem note from the ground.

The investigation of plant type, days to 50 % silking, plant height, ear height, tassel branch number and stalk diameter had been carried out in each backcross generation. The genotypes of the plants in each backcross had been identified by the functional marker and the flanking markers. Few plants differing obviously from the recurrent parent lines had been discarded to recover the genetic background of recipient parent. These lines with favorable allele of *ZmVTE4* had been selected based on their genotypes.

Tocopherol extraction and quantification

Tocopherol extraction and quantification by high performance liquid chromatography (HPLC) was performed according to Egesel et al. (2003) at National Maize Improvement Center of China, China Agricultural University. Tocopherols were separated on a reverse phase C_{30} column (5 μm particle,

4.6 mm × 25 cm), kept at 30 °C and analyzed by scanning from 200 to 800 nm, with a 2-nm bandwidth and a photodiode-array (PDA) detector connected to Shimadzu HPLC equipment. Detection at 295 nm and quantification of tocopherols was performed using external standards and six-point standard curves. Tocopherol peaks in each sample were identified on the basis of co-chromatography, retention times, and by comparing absorption spectra on the photodiode array detector with those of authentic standards. Commercial standards for α-tocopherol, γ-tocopherol, and δ-tocopherol were purchased from Sigma (St Louis, MO, USA). Other chemicals (technical grade or higher) and chromatography solvents (HPLC-grade) used in this study were bought from Sigma or Merck (Darmstadt, Germany). The total tocopherol content of a sample was computed by summing the amounts of individual tocopherols (α, γ and δ-tocopherols). Tocopherol composition was expressed as the α-tocopherol/γ-tocopherol ratio.

Data analysis

The score of each band was recorded according to the method of MAPMAKER (Lander et al. 1987). The percentage of recurrent parent genome content had been computed by the formula G(g) = [L + X(g)]/(2L) (Jun-Hong and Yong-Lian 2002). The G(g) specifies the percentage of recurrent parent genome content in g backcross generation. X(g) specifies the number of markers with the same genotypes as the recipient parent lines in g backcross generation. L specifies the number of total markers. The total tocopherol content of a sample was computed by summing the amounts of individual tocopherols (α, δ and γ-tocopherols). Analysis of variance (ANOVA) was performed for each compound in five selected converted lines and their recurrent parent using the PROC GLM routine of SAS (SAS Institute 1988).

Results

Parental polymorphism analysis between four sweet corn lines and SY999

The functional markers, *InDel118* exhibited codominant polymorphism between 4 sweet corn lines and SY999 and no polymorphism had been detected by *InDel7*. A band of approximately 373 bp DNA fragment was observed in the PCR amplified product of SY999 and a band of 491 bp DNA fragment was observed in 4 sweet corn lines. These two bands were clearly distinguished (Fig. 1a). Two SSR markers, *bnlg1237* and *phi085* also exhibited codominant polymorphism between 4 sweet corn lines and SY999.

Foreground selection in backcross and selfed generation

In the process of M01 conversion, the functional marker *InDel118* identified 63 heterozygous individuals in BC_1F_1 generation, 43 heterozygous plants of BC_2F_1 generation, 33 heterozygous plants in BC_3F_1 generation and 18 homozygotes at the *ZmVTE4* locus in BC_3F_2 generation (Fig. 1b). The same identification by the functional marker *InDel118* had been carried out in the process of M14, K140 and K185 conversion and 7–15 homozygotes at the *ZmVTE4* locus were selected in BC_3F_2 generation. The standard Chi square test for goodness of fit showed that the marker segregated according to the expected Mendelian ratio of 1:1 for each backcross generation (Table 1). The value of Chi square ranged from 0.04 to 1.06 and all of them were less than 3.84. Three to five plants heterozygous for the *ZmVTE4* locus were single recombinants on either side of *ZmVTE4*, while no double recombinants were identified in BC_1 generation. In BC_3F_2 generation, these selected plants were double recombinants and homozygous at the flanking marker with recurrent parent lines.

Background selection in BC_3F_2 generation

The average recurrent parent genome content of the M01-BC_3F_2 generation was 95.85 % with a range of 92.25–99.30 % (Table 2). All individuals exceeded the expected recurrent parent genome content (93.75 %) except for M01-32 and M01-56. The individuals with the maximal proportion of the donor genome content were M01-32, M01-44 and M01-56. The average donor genome content was 1.25 %, the average heterozygous genome content was 2.27 % and the average proportion of unexplained variation (either due to unknown allele type or missing data) was 0.62 %. In the BC_3F_2 progenies of M14, K140 and K185, the ranges of recurrent parent genome content were 91.78–96.58 %, 91.78–97.26 % and

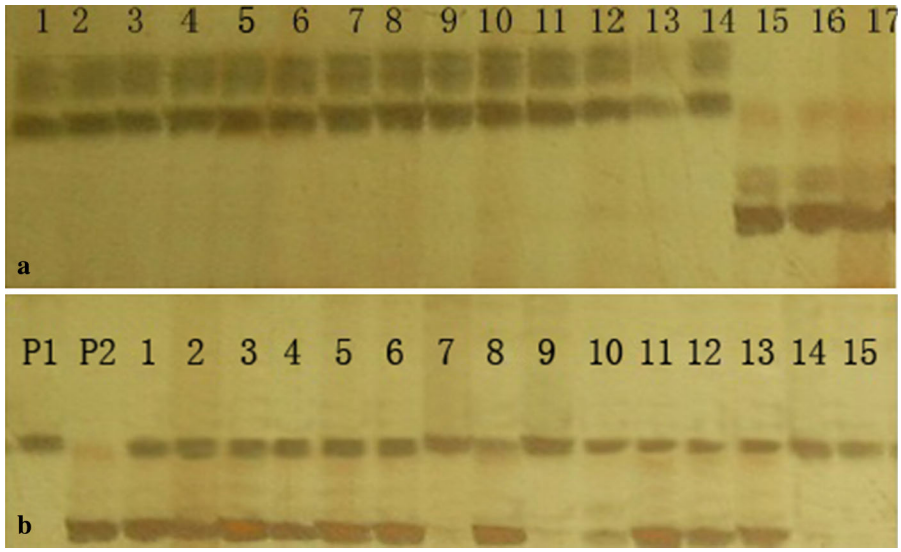

Fig. 1 **a** Parental polymorphism analysis using *ZmVTE4* functional marker, *InDel118* between SY999 and sweet corn lines. *Lanes 1–3* M01, *4–6* M14, *7–9* K140, *10–14* K185, *15–17* SY999. **b** Foreground selection for identification of recessive homozygotes for *ZmVTE4* using *InDel118* in the BC_3F_2 population of M01 × SY999. *P1* M01, *P2* SY999, *Lanes 1–15* BC_3F_2 individuals

Table 1 The Chi square test of the functional marker (*InDel118*) in three backcross segregations from 4 elite sweet corn lines

Recurrent parent	Generation	No. of plants	*InDel118*		x_c^2 (1:1)	$x_{0.05,1}^2$
			373 bp	491 bp		
M01	BC_1F_1	130	63	67	0.12	3.84
	BC_2F_1	88	43	45	0.05	
	BC_3F_1	72	33	39	0.50	
M14	BC_1F_1	125	60	65	0.20	
	BC_2F_1	95	49	46	0.09	
	BC_3F_1	67	37	30	0.73	
K140	BC_1F_1	70	38	32	0.51	
	BC_2F_1	80	43	37	0.45	
	BC_3F_1	97	46	51	0.26	
K185	BC_1F_1	100	49	51	0.04	
	BC_2F_1	78	42	36	0.46	
	BC_3F_1	84	44	40	0.19	

91.89–97.30 %, with a mean of 93.97, 94.41 and 94.83 %, respectively.

Phenotypic selection in BC_2F_1 and BC_3F_1

The investigation of plant vigour, days to 50 % silking, plant height, ear height, tassel branch number and stalk diameter was carried out in each backcross generation. Few individuals differing from the recurrent parent lines had been discarded in the BC_1F_1 and BC_2F_1 generation. The difference of agronomic traits between the progenies and their recurrent parent lines had been detected by T-test (Table 3). The T-values of the four agronomy traits in BC_2F_1 ranged from 0.04 to 3.53 with a mean of 1.08. No significant differences were detected between the BC_2F_1 generation and the recurrent lines except for the ear height of the K185 and its BC_2F_1 generation. In the progenies of BC_3F_1, the T-values of the four agronomy traits ranged from 0.12 to 1.90 with a mean of 0.82. There is no

Table 2 Recurrent and donor parent genome content of 18 selected M01-BC_3F_2 individuals revealed by marker aided background analysis

Individual plant no.	Recurrent parent genome content (%)	Donor parent genome content (%)	Heterozygous genome content (%)	Unexplained variation (%)
M01-1	95.07	1.41	2.11	1.41
M01-2	96.48	1.41	2.11	0
M01-5	95.77	1.41	2.82	0
M01-15	99.30	0	0.70	0
M01-19	97.89	0	0.70	1.41
M01-27	97.18	0	1.41	1.41
M01-30	96.48	1.41	2.11	0
M01-32	93.66	2.82	3.52	0
M01-34	95.07	1.41	3.52	0
M01-44	95.07	2.82	2.11	0
M01-45	94.36	1.41	2.82	1.41
M01-51	96.48	0	3.42	0
M01-53	95.77	1.41	2.82	0
M01-56	92.25	2.82	2.11	2.82
M01-57	97.18	1.41	1.41	0
M01-62	96.48	0	2.11	1.41
M01-70	95.77	1.41	2.82	0
M01-72	95.07	1.41	2.11	1.41

significant difference between the progenies and their recurrent parent lines.

These homozygotes with *InDel7* (deletion) and *InDel118* (deletion) sites on the *ZmVTE4* gene were selected from the progenies of BC_3F_2 and their seeds were threshed by hand to quantify the tocopherols content. The numbers of selected plants from the converted BC_3F_2 families of M01, M14, K140 and K185 were 18, 11, 15 and 13, respectively. Five selected families with higher total tocopherols per backcross progenies and their recurrent parents were sown in randomized complete block design to quantify the tocopherol components (Table 4). The results showed that the converted lines with homozygous *ZmVTE4* gene improved significantly the content of γ-tocopherol and total tocopherols over the recipient lines. The average γ-tocopherol content in the progenies of M01, M14, K140 and K185 were 40.31, 62.42, 26.98 and 76.07 μg/g, respectively. The γ-tocopherol contents of the four progenies increased by 16.05, 28.15, 18.92 and 26.22 μg/g, respectively. The average total tocopherol content in the progenies of M01, M14, K140 and K185 were 54.32, 67.70, 43.32 and 88.00 μg/g, with the increments of 21.03, 28.46, 26.76 and 30.57 μg/g, respectively. The α-tocopherol content of K140 and K185 converted lines increased significantly and no difference was detected between the M14 converted lines and their recurrent line. The average α-tocopherol content of K140 and K185 were 14.58 and 9.06 μg/g, with the increments of 7.73 and 5.33 μg/g, respectively. The α/γ-tocopherol ratio showed decreasing trend in these converted lines except for K185.

Discussion

Vitamin E is a kind of indispensable micronutrient acquired by humans and animals mainly from plants. Sweet corn is harvest at immatured stage as vegetable and its consumption is growing (Luo et al. 2014). The enhancement of tocopherols in maize is restricted because of expensive cost and labor intensity measured by HPLC. It is a good issue to breed by marker assisted selection and we were lucky to use the functional marker of *ZmVTE4* in sweet corn.

In this study, one functional marker within the *ZmVTE4* and two flanking SSR markers exhibited the

Table 3 The comparison of agronomic traits between the progenies of BC_2F_1, BC_3F_1 and their corresponding recurrent parent lines by T-test

Materials	Agronomy traits	Recurrent line	BC_2F_1			BC_3F_1		
		Mean ± se	Mean ± se	T value	P value	Mean ± se	T value	P value
M01	Plant height (cm)	149.55 ± 7.89	167.45 ± 6.61	1.67	0.10	164.70 ± 4.67	1.57	0.12
	Ear height (cm)	40.02 ± 3.67	39.55 ± 5.90	0.18	0.86	42.39 ± 12.71	0.93	0.36
	No. of tassel branch	8.54 ± 1.12	9.06 ± 3.35	0.72	0.48	8.59 ± 3.35	0.12	0.91
	Stalk diameter (cm)	1.36 ± 0.083	1.39 ± 0.27	1.98	0.06	1.39 ± 0.27	1.90	0.07
M14	Plant height (cm)	137.33 ± 5.03	150.85 ± 6.11	1.4	0.18	142.86 ± 7.34	0.54	0.59
	Ear height (cm)	35.67 ± 1.53	35.08 ± 8.82	0.11	0.91	33.81 ± 6.36	0.34	0.74
	No. of tassel branch	9.33 ± 0.58	8.46 ± 2.16	0.73	0.48	8.11 ± 3.13	0.67	0.51
	Stalk diameter (cm)	1.73 ± 0.15	1.75 ± 0.21	0.10	0.92	1.71 ± 0.20	0.19	0.85
K140	Plant height (cm)	153.80 ± 6.18	155.2 ± 6.19	0.24	0.82	159.75 ± 5.63	0.83	0.41
	Ear height (cm)	54.60 ± 2.97	48.40 ± 4.10	1.37	0.19	49.22 ± 9.29	1.48	0.15
	No. of tassel branch	6.60 ± 2.51	4.40 ± 1.17	1.48	0.16	4.56 ± 2.35	1.72	0.09
	Stalk diameter (cm)	1.66 ± 0.11	1.52 ± 0.19	1.52	0.15	1.59 ± 0.19	0.78	0.44
K185	Plant height (cm)	165.17 ± 8.26	162.75 ± 7.83	0.38	0.71	167.76 ± 8.88	0.33	0.74
	Ear height (cm)	49.17 ± 4.45	37.81 ± 4.33	3.53**	0.01	40.46 ± 9.45	0.77	0.45
	No. of tassel branch	8.17 ± 1.33	6.63 ± 2.09	1.67	0.11	7.10 ± 3.16	0.81	0.42
	Stalk diameter (cm)	1.42 ± 0.10	1.48 ± 0.16	0.94	0.36	1.53 ± 0.24	1.18	0.24

** significant difference at $P \leq 0.01$

polymorphism between the 4 sweet corn lines and the donor parent lines. As these three markers exhibited codominant polymorphism, we could identify homozygous and heterozygous genotypes for the *ZmVTE4* gene. The individual plants in any segregating population could be scored directly on the functional marker. Identification of heterozygotes at the seedling stage in the process of backcross could reduce the waste of labor and material resources.

The linkage drag is a main problem in a backcrossing breeding program (Frisch et al. 1999). In this study, two flanking markers, *bnlg1237* and *phi085* were positioned at 10.1 and 11.9 cM respectively from the *ZmVTE4* gene (Chander et al. 2008). Ribaut et al.(2002) found that flanking markers as close as 2 cM is considered the ideal option, while transferring the same target gene from elite to elite lines, the flanking marker at more distance might be more effective. SY999 is an elite common corn inbred line with the favorable allele of *ZmVTE4*. The flanking markers with relatively far genetic distance will obtain the various progenies.

The objective of the background/whole genome selection is to recover rapidly maximum proportion of recurrent parent genome at non-target loci through markers that are distributed evenly throughout the whole genome (Babu et al. 2005). The ample SSR anchored markers in maize genome make it possible to be applied to genetic background selection. However, it is still expensive to employ background selection by SSR in each backcross generation. In order to reduce the cost of breeding, the combination of agronomic traits selection and marker aided background selection had been performed in this study. Few plants were rejected in the BC_1 and BC_2 generation and marker aided background selection was carried out only in the BC_3 generation. The average recurrent parent genome contents among 4 progenies population of BC_3F_1 were more than the expected value (93.5 %). The results showed that the phonotypic selection was helpful to recovery rapidly the genetic background of recipient parents. Ribaut et al. (2002) predicted that the selection response for background analysis depends on the recombination frequency between the target gene and the flanking markers and on the densities of markers on the carrier and non-carrier chromosomes. The number and distribution of non-target markers decided the efficiency of background selection. In our

Table 4 The comparison of tocopherol components content (μg/g dry weight) among the 4 converted lines and their corresponding recurrent parent lines

Parent/ progeny	δ-Tocopherol	γ-Tocopherol	α-Tocopherol	Total tocopherol	α/γ	Recurrent parent genome content (100 %)
SY999 (donor parent)	4.73 ± 0.12	59.72 ± 1.70	1.77 ± 0.05	65.41 ± 0.99	0.03 ± 0.00	
M01 (recurrent parent)	1.68 ± 0.05c	24.26 ± 1.57b	9.26 ± 0.79c	33.29 ± 0.72b	0.37 ± 0.02a	
M01-02	2.49 ± 0.27b	40.51 ± 0.68a	10.46 ± 0.31bc	53.45 ± 1.27a	0.26 ± 0.00b	95.77
M01-27	2.09 ± 0.04bc	39.76 ± 3.96a	11.15 ± 1.65abc	50.5 ± 3.15a	0.28 ± 0.01b	95.07
M01-44	2.32 ± 0.17b	41.12 ± 0.42a	11.94 ± 0.06ab	54.61 ± 0.96a	0.29 ± 0.00b	97.89
M01-70	3.91 ± 0.24a	43.27 ± 0.57a	12.32 ± 0.18ab	59.49 ± 0.64a	0.28 ± 0.01b	96.48
M01-72	3.51 ± 0.14a	36.91 ± 0.06a	13.11 ± 0.25a	53.53 ± 0.45a	0.36 ± 0.01a	95.07
M14 (recurrent parent)	1.91 ± 0.08ab	34.27 ± 1.08b	3.14 ± 0.85a	39.24 ± 0.64b	0.09 ± 0.00a	
M14-18	1.52 ± 0.08b	63.78 ± 1.77a	3.13 ± 0.14a	68.43 ± 1.71a	0.05 ± 0.00b	95.89
M14-20	3.00 ± 0.69a	63.43 ± 1.26a	3.70 ± 0.09a	70.13 ± 1.86a	0.06 ± 0.00ab	93.05
M14-38	2.13 ± 0.18ab	61.52 ± 1.82a	2.81 ± 0.79a	66.46 ± 1.21a	0.05 ± 0.01b	93.22
M14-77	2.13 ± 0.08ab	62.16 ± 2.63a	2.18 ± 0.20a	67.53 ± 1.68a	0.04 ± 0.00b	93.22
M14-80	1.58 ± 0.13b	61.22 ± 0.43a	3.12 ± 0.62a	65.97 ± 0.00a	0.05 ± 0.01b	96.58
K140 (recurrent parent)	1.50 ± 0.07a	8.06 ± 0.22b	6.85 ± 1.31b	16.56 ± 1.33b	0.89 ± 0.04a	
K140-10	1.42 ± 0.33a	28.71 ± 1.70a	13.20 ± 1.74a	43.33 ± 0.29a	0.46 ± 0.09b	96.58
K140-55	1.58 ± 0.63a	26.07 ± 0.86a	15.70 ± 0.96a	43.36 ± 2.46a	0.60 ± 0.02b	95.21
K140-73	2.26 ± 0.11a	24.80 ± 0.53a	14.23 ± 1.66a	41.29 ± 2.30a	0.57 ± 0.05b	94.52
K140-81	1.57 ± 0.41a	29.22 ± 1.81a	15.99 ± 0.35a	46.79 ± 2.57a	0.55 ± 0.02b	95.89
K140-87	1.93 ± 0.87a	26.12 ± 1.79a	13.78 ± 1.19a	41.83 ± 3.85a	0.53 ± 0.01b	94.52
K185 (recurrent parent)	2.90 ± 0.06a	49.85 ± 1.03b	3.43 ± 0.73b	57.43 ± 3.03b	0.07 ± 0.01b	
K185-06	3.36 ± 0.24a	74.92 ± 0.69a	10.48 ± 1.19a	88.77 ± 0.25a	0.14 ± 0.02a	94.59
K185-08	2.22 ± 0.29a	75.66 ± 3.91a	9.55 ± 0.94a	87.43 ± 4.56a	0.13 ± 0.01a	97.30
K185-37	2.29 ± 0.40a	79.46 ± 2.55a	8.05 ± 0.62a	89.79 ± 2.77a	0.10 ± 0.00ab	96.62
K185-44	3.13 ± 0.68a	76.31 ± 0.90a	8.44 ± 1.18a	87.88 ± 0.96a	0.11 ± 0.02ab	96.62
K185-77	3.39 ± 0.16a	74.00 ± 2.22a	8.76 ± 0.21a	86.15 ± 1.85a	0.12 ± 0.01a	95.95

Means with different letters within each converted line indicate significant differences at $P \leq 0.05$

study, we chose markers located evenly on the whole maize genome according to the public consensus map. So the recurrent parent genome content is unbiased sampling and estimating.

The *ZmVTE4* allele is codominant and was significantly associated with α-tocopherol levels in maize kernels (Li et al. 2012). In their research, the haplotypes of the two *InDels* could explain 33 % of α-tocopherol variation and was not associated with the γ-tocopherol and total tocopherol concentration. However, in our study, the α-tocopherol levels increased in three backcross converted lines except for M14. M14 was the only inbred line with white kernels and the relationship between the kernel color and the α-tocopherol levels was unclear. It is notable that the γ-tocopherol and total tocopherol concentration increased in all four backcross converted sweet corn lines. A few main QTLs associated with tocopherols had been detected on chromosome 5. Rocheford et al. (2002) detected two chromosome regions (bins 5.3 and 5.5), which were associated with γ-tocopherol and α-tocopherol. Wong et al. (2003) detected two chromosome intervals, 16–30 and 49–103 cM on chromosome 5, associated with α-tocopherol, γ-tocopherol, and δ-tocopherol and total tocopherols. The interval of 67–112 cM on chromosome 5 was associated with γ-tocopherol and total tocopherol (Feng et al. 2013). In our study, the highest

tocopherols content lines were not these lines with the highest proportion of recurrent parent genome. It indicated that *ZmVTE4* had different expression in the sweet corn genetic background or the non-target genome introgressing into the recipient parent lines affected the content of tocopherols.

The rapid introgression strategy in this investigation was characterized by both marker aided and phenotypic selection. The functional marker made it possible to fix the target gene, the SSR markers used in background selection accelerated the recovery of recipient parent genome, and the agronomic trait selection in early generations was useful to speed up the recovery of recurrent parent genome. The converted lines with high tocopherols content and high recurrent parent genome content were obtained in the BC_3F_2 generation. With the development of third generation marker technology such as SNPs and the association analysis, the power and efficiency of marker assisted selection is expected to improve in future. The breeding by molecular design will become true.

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