RESEARCH NOTE

Complementation of sweet corn mutants: a method for grouping sweet corn genotypes

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Introduction

Maize mutants that alter the composition of endosperm starch and are consumed as sweet corn or corn-derived vegetable crops have more kernel sweetness than the normal maize. Allelic complementation between mutant gene(s) leading to normal kernel in hybrid is concern for diverse endosperm mutant's deployment. Based on F_1s kernel phenotype and segregation of F_2s derived using normal corn genotype V373, 16 sweet corn genotypes and two testers were determined to possess a single recessive mutant gene. Consequently, allelic relationship was investigated using complementation of mutant gene in VSL1 and VL15 with 16 diverse genotypes. From first set of 16 test crosses with VSL1, VLS11 × VLS1 and HKI 1831 × VLS1 exhibited complementation in F_1s and segregation in F_2s while in second set with VL 15, complementation in F_1s and segregation in F_2s was observed in all test crosses except VLS11 × VL15 and HKI 1831 × VL15. Thus, kernel phenotype revealed that normal kernels appeared due to complementation of dissimilar mutants whereas noncomplementation of similar mutants leads to sweet corn kernels in hybrids. We further hypothesized that kernel phenotype-based genetic complementation is a simple tool and can be used efficiently in grouping of endosperm mutants.

Sweet corn is considered as a corn-derived vegetable crop developed through recessive mutations having high sugar content at milky stage (Tracy 1997). The markets for sweet corn are now expanding and the demands are increasing due to urbanization and increase in purchasing power (Lertrat and Pulam 2007). Consequently, the cultivation of sweet corn is also expanding in many nontraditional countries across the continents including India. Lack of well-adapted cultivars to the diverse cropping conditions of sub-tropical/tropical regions of India is amongst one of the factors that is responsible for limited production and productivity of sweet corn.

Maize endosperm mutant genes that affect quality of sweet corn can be grouped in two classes. One group of mutants namely *brittle1* (*bt1*), *brittle2* (*bt2*) and *shrunken2 (sh2)* accumulate sugars at the expense of starch and have low total carbohydrate at the mature kernel stage (Boyer and Shannon 1984). At 18–21 days after pollination (harvest stage of sweet corn), these mutants have four to eight times higher total sugar than the normal corn (Holder *et al.* 1974). Due to comparative high sugar level, this group of mutants can be used even alone in development of sweet corn varieties/hybrids and are often called super sweet or extra sweet. Another group of mutants, namely *amylose extender1* (*ae1*), *dull1* (*du1*), *sugary 1* (*su1)* and *waxy1* (*wx1*), alter the types and amount of polysaccharides produced in endosperm. The mutant *ae1*, *du1* and *wx1* generally results in slightly less starch content in the mature kernel than normal types (Boyer and Shannon 1984). Compared to first group of mutants, these three mutants result in a smaller increase in total sugar content at 18–21 days after pollination and do not make acceptable sweet corn when used singly. However, double or triple combinations of second group of mutants result in sugar levels equal to those found in first group of mutants (Creech 1966).

Many of the mutant genes identified early in the genetic analysis of corn were easily identifiable based on kernel phenotype, and the genetic segregation could be observed on a single F_2 cob. Essentially all these mutants were recessive and required the homozygous allelic state to express the altered endosperm composition. Further, many new mutants

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were identified with phenotypes similar to known mutants however, genetic analysis indicates that they are genotypically different. Therefore, a test of genetic complementation is essential to determine whether a new mutant is allelic to existing one or novel and nonallelic (Coe 1985). The test, therefore, helps in grouping endosperm mutants for development of single or multiple mutants-based sweet corn hybrids. Recently, SSR markers linked with *su1* and *sh2* genes have been identified using biparental mapping populations (Hossain *et al.* 2015). This may facilitate the grouping of sweet corn genotypes; however, markers derived from biparental population may have limited application in analysing mutants with multiple alleles and alleles from diverse genetic background. Thus, the concept of complementation, developed by Lewis in *Drosophila* and further used by Benzer in T4 phage to group mutants (Strickberger 1968), has vital application in grouping germplasm for developing sweet corn hybrids. The present investigation was, therefore, planned with the objective to validate the type of sweet corn mutant genes through complementation test based on kernel phenotype and to group the sweet corn genotypes for hybrid development programme.

Materials and methods

All the experiments were conducted at research farm, Vivekanand Parvatiya Krishi Anusandhan Sansthan, Hawalbagh, Almora (Uttarakhand) and Winter Nursery Centre, Directorate of Maize Research, Hyderabad. Two sweet corn genotypes, namely VL15, a sugary mutant (*su1su1*) and VSL1, a shrunken mutant (*sh2sh2*) were test crossed with a set of 16 sweet corn genotypes to generate 32 test crosses. Kernels of each F_1 test cross were phenotyped visually, and grouped into normal corn (NC) and modified endosperm type sweet corn (SC). The F_1 plants of each cross were selfed to generate F_2 kernels and were phenotyped visually into NC and SC categories. Assumption was made that mutant at same locus in both the parents will not complement to each other with realization of mutant phenotype (SC) in F_1 as well as in F_2. On the other hand, mutation at one locus in one parent and at another locus in second parent will complement each other with normal kernel in F_1 but segregation in F_2 in the ratio of nine normal (*Su1_Sh2_*) and seven sweet corn (*Su1_sh2sh2*, *su1su1Sh2_* and *su1su1sh2sh2*) kernel. Before attempting test crosses, all the 16 sweet corn genotypes and the testers were validated for presence of a single endosperm mutant gene in recessive condition. To ensure this, all the 16 sweet corn genotypes were crossed with a normal corn genotype, V373. The F_1s and F_2s kernels were phenotyped and scored as NC or SC. Again the assumption was made that F_1 kernels of a cross between SC and NC will be normal while F_2 kernels will be expected to segregate in the ratio 3 NC : 1 SC, if the single recessive mutant gene is conferring modified endosperm. Further, both testers were also crossed with each other and also with normal corn genotype V373. The F_1 and F_2 kernels were phenotyped and scored as NC or SC to determine the number of mutant genes present in testers. Controlled pollination was adopted to generate F_1 and F_2 kernels. Dried cobs were harvested; kernels were shelled off and used for recording observation as number of NC or SC individually in each F_1s and F_2s. Chi square (χ^2) test was applied to accept goodness of fit of observed ratio with expected ratio in segregating generation (Snedecor and Cochran 1989).

Results and discussion

Application and utilization of heterotic patterns in maize had significant influence on yield improvement, efficient testing of hybrids, and increasing the probability of identifying desirable hybrids (Tracy 1990). Such heterotic grouping is not well established in sweet corn and is one of the main reasons behind its narrow genetic variability. In

Table 1. Test cross to validate number and nature of mutant gene in sweet corn lines.

Test cross (SCG × NCG)	F_1 kernel phenotype	Observed F_2 kernel phenotype		χ^2 value	*P* value
		SC	NC		
VSL2 × V373	Normal	163	61	0.595	0.440
VSL3 × V373	Normal	191	62	0.033	0.856
VSL4 × V373	Normal	171	63	0.462	0.497
VSL5 × V373	Normal	181	67	0.538	0.463
VSL6 × V373	Normal	211	66	0.203	0.652
VSL7 × V373	Normal	207	73	0.171	0.679
VSL8 × V373	Normal	222	79	0.249	0.618
VSL9 × V373	Normal	232	86	0.709	0.400
VSL10 × V373	Normal	205	75	0.476	0.490
VSL11 × V373	Normal	215	73	0.019	0.892
VSL12 × V373	Normal	198	73	0.542	0.461
VSL14 × V373	Normal	228	80	0.156	0.693
VSL15 × V373	Normal	198	69	0.101	0.750
VSL16 × V373	Normal	183	69	0.762	0.383
VSL17 × V373	Normal	159	58	0.346	0.557
HKI 1831 × V373	Normal	175	50	0.926	0.336

addition to heterotic grouping, sweet corn, unlike normal corn, also requires to be grouped based on allelic relationship of mutant genes responsible for kernel modification and higher sweetness. So, sweet corn genotypes developed using established or novel mutant alleles need to be grouped based on allelic relationship for further utilization in hybrid/variety development.

To validate the presence of single recessive mutant gene in 16 sweet corn genotypes, test crosses were made with normal corn genotype (V373) with corresponding sweetness alleles in dominant form (table 1). The phenotypic observation of F_1 kernels proved the identity of lines that they carry recessive mutant allele for sweetness. The F_1 plants of each cross were raised separately and selfed through controlled pollination to generate F_2 kernels and were phenotyped visually as normal and sweet corn types. The observed phenotypic classes of each F_2 kernels were validated using χ^2 test with the expected classical monogenic segregation ratio of 3 : 1 (table 1). The χ^2 analysis accepted that each F_2 had segregation ratio of three NC kernels to one SC kernel and therefore, goodness of fit was observed between observed and expected ratios. The acceptance of 3:1 ratio in F_2 kernels of a cross between sweet corn and normal maize genotype confirms that each sweet corn genotype had a single recessive mutant gene for kernel modification.

Both the testers, namely, VL15 and VSL1 were crossed to each other and also crossed with normal corn genotype (V373) to determine allelic relationships among the mutant gene present in testers and also with allele present in normal maize genotype (table 2). Normal phenotype of F_1 kernels was noticed in both, tester by tester cross and testers by normal corn genotype cross (table 2). This indicates complementation of mutant phenotype of sweet corn by the corresponding dominant allele from the normal maize genotype / other sweet corn genotype. Monogenic segregation ratio of three normal and one sweet corn type kernels was observed in F_2 derived from the crosses between V373 and testers (table 2). In case of tester by tester cross, F_1 kernels were phenotypically normal indicating complementation of mutant gene present in one tester by corresponding dominant allele present in second tester (figure 1). This indicates that both the testers have different endosperm mutant genes. Selfing of tester by tester F_1 hybrids gave F_2 kernels with normal and sweet corn-type phenotypes. Validation of observed phenotypic ratio of F_2 population with χ^2 test indicates goodness of fit with expected classical digenic phenotypic ratio of 9 NC : 7 SC. Digenic segregation pattern in F_2 further supports the assumption that single mutant gene present in each tester are different from each other.

Phenotype of the kernels derived from a biparental cross is influenced by the alleles present in both the parents (xenia effect). Considering the immediate allelic effect on kernels, each F_1 ear of 32 test cross hybrids developed by crossing 16 sweet corn genotypes with two testers was phenotypically observed and the kernels were classified into four classes: (i) F_1s with sweet corn kernels when crossed with VSL1,

Table 2. Test cross to validate number and nature of mutant gene in sweet corn testers.

	VL15					VSL1				
Testers	F_1 phenotype	Expected segregation ratio in F_2	Observed segregation in F_2	χ^2 value*	*P* value	F_1 phenotype	Expected segregation ratio in F_2	Observed segregation in F_2	χ^2 value*	*P* value
VL15	–	–	–	–	–	NC	9:7	146 : 123 (NC : SC)	0.426	0.514
VSL1	NC	9:7	169 : 136 (NC : SC)	0.087	0.767	–	–	–	–	–
V373	NC	3:1	161 : 51 (NC : SC)	0.101	0.751	NC	3:1	222 : 78 (NC : SC)	0.160	0.689

*$\chi^2 < 3.84$ is nonsignificant at 0.05 probability level; NC, normal corn; SC, sweet corn.

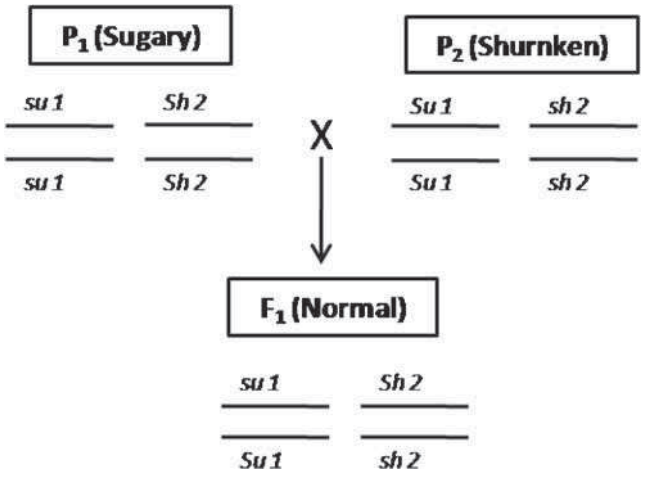

Figure 1. Mode of complementation between sweet corn mutants.

(ii) F_1s with sweet corn kernels when crossed with VL15, (iii) F_1s with normal kernels when crossed with VSL1 and (iv) F_1s with normal kernel when crossed with VL15. Of the 16 test crosses, where VSL1 was used as tester, F_1 kernels of two cross combinations, namely, VSL11 × VSL1 and HKI1831 × VSL1 produced NC kernel, whereas the remaining 14 test crosses had modified SC endosperm phenotype (table 2). On the other hand, out of 16 test crosses derived using VL 15, two crosses, namely, VSL11 × VL15 and HKI1831 × VL15 possessed modified sweet corn kernel phenotype. The remaining 14 cross combinations with VL15 produced normal kernels. The F_1 with normal kernels indicated the case of complementation since mutants for kernel modification are expected to be at different locus in parents and corresponding dominant alleles did not allow mutant alleles to express and modify endosperm when present in heterozygous condition (figure 1). Such combinations, therefore, cannot be used in sweet corn hybrid development programme. In other cases, where F_1 exhibited mutant phenotype indicates that both the parents have mutation in the same gene i.e. both the parents have same allele. Consequently, such mutant combinations are unable to complement each other and modified SC kernels are observed on hybridization. Further, each F_1 was advanced to F_2 through controlled pollination to further validate the allelic relationships among the parents. The F_1s with sweet corn kernels produced similar kernels in F_2. However, F_2 kernels derived from the F_1s with normal kernel had both normal as well as

Table 3. Complementation test of sweet corn lines using sweet corn testers.

Sweetcorn genotypes	Tester	Observed F_1 kernel type	Observed segregation of F_2 kernels	χ^2 value*	*P* value
VSL2	VSL1	Sweet corn	All sweet corn	0.000	1.000
VSL3	VSL1	Sweet corn	All sweet corn	0.000	1.000
VSL4	VSL1	Sweet corn	All sweet corn	0.000	1.000
VSL5	VSL1	Sweet corn	All sweet corn	0.000	1.000
VSL6	VSL1	Sweet corn	All sweet corn	0.000	1.000
VSL7	VSL1	Sweet corn	All sweet corn	0.000	1.000
VSL8	VSL1	Sweet corn	All sweet corn	0.000	1.000
VSL9	VSL1	Sweet corn	All sweet corn	0.000	1.000
VSL10	VSL1	Sweet corn	All sweet corn	0.000	1.000
VSL11	VSL1	Normal corn	146 : 105 (NC : SC)	0.375	0.540
VSL12	VSL1	Sweet corn	All sweet corn	0.000	1.000
VSL14	VSL1	Sweet corn	All sweet corn	0.000	1.000
VSL15	VSL1	Sweet corn	All sweet corn	0.000	1.000
VSL16	VSL1	Sweet corn	All sweet corn	0.000	1.000
VSL17	VSL1	Sweet corn	All sweet corn	0.000	1.000
HKI1831	VSL1	Normal corn	152 : 112 (NC : SC)	0.189	0.664
VSL2	VL15	Normal corn	185 : 114 (NC : SC)	0.394	0.530
VSL3	VL15	Normal corn	174 : 109 (NC : SC)	0.170	0.680
VSL4	VL15	Normal corn	195 : 140 (NC : SC)	0.522	0.470
VSL5	VL15	Normal corn	137 : 123 (NC : SC)	0.239	0.625
VSL6	VL15	Normal corn	160 : 140 (NC : SC)	0.364	0.546
VSL7	VL15	Normal corn	171 : 114 (NC : SC)	0.353	0.552
VSL8	VL15	Normal corn	220 : 140 (NC : SC)	0.395	0.530
VSL9	VL15	Normal corn	163 : 101 (NC : SC)	0.151	0.697
VSL10	VL15	Normal corn	214 : 155 (NC : SC)	0.456	0.499
VSL11	VL15	Sweet corn	All sweet corn	0.000	1.000
VSL12	VL15	Normal corn	207 : 120 (NC : SC)	0.286	0.593
VSL14	VL15	Normal corn	132 : 119 (NC : SC)	0.003	0.957
VSL15	VL15	Normal corn	184 : 113 (NC : SC)	0.701	0.402
VSL16	VL15	Normal corn	172 : 113 (NC : SC)	0.506	0.477
VSL17	VL15	Normal corn	162 : 118 (NC : SC)	0.294	0.588
HKI1831	VL15	Sweet corn	All sweet corn	0.000	1.000

*$\chi^2 < 3.84$ is nonsignificant at 0.05 probability level; SC, sweet corn; NC, normal corn.

Table 4. Complementation test to determine genetic constitution of sweet corn mutants.

Unknown SC line	F_1 phenotype with *su1* mutant tester	F_1 phenotype with *sh2* mutant tester	Expected genotype of unknown line
1	Normal corn	Sweet corn	*Su1Su1sh2sh2*
2	Sweet corn	Normal corn	*su1su1Sh2Sh2*
3	Normal corn	Normal corn	*Su1Su1Sh2Sh2**
4	Sweet corn	1 : 1 (NC : SC)	*su1su1Sh2sh2*
5	1 : 1 (NC : SC)	Sweet corn	*Su1su1sh2sh2*

*Homozygous recessive mutant for 3rd sweetness gene, can serve as tester for 3rd gene.

modified SC kernels. The number of normal and modified kernels were counted in each segregating F_2s (table 3) and were analysed using χ^2 test to determine goodness of fit with digenic segregation ratio of 9 : 7, since each parent had a mutant gene different from other parent. The χ^2 test revealed concurrence of observed normal and modified SC kernels ratio in F_2 population of VSL11 × VSL1 (146 : 105) and HKI 1831 × VSL1 (152 : 112) with expected ratio of 9 : 7 (table 3). Similarly, the F_2 ratio of normal and modified kernels in 14 cross combinations with VL15 had goodness of fit with the expected ratio of 9 : 7. The results indicated that nonsegregating sweet corn populations developed using either VSL1 or VL15 as tester had similar alleles in both the parents, therefore exhibited modified SC kernels in both F_1 and F_2. On the other hand, the crosses with segregation pattern of 9 : 7 NC and SC kernels in F_2 from normal F_1 kernels indicates that mutant alleles present in both the parents are nonallelic. Consequently, complementation occurred in F_1 and segregation pattern of normal and sweet corn were observed in the F_2 kernels. Determination of such allelic relationships is critical in sweet corn development programme. In India as well as abroad, maize breeders are developing sweet corn inbred genotypes continuously through conversion of normal maize genotypes into sweet corn or through hybridization followed by standard procedures of inbred genotype development. To determine the allelic relationship among newly developed genotypes, the complementation studies using simple criteria of appearance of the F_1 kernels seems to be rewarding and easy approach. Based on the phenotypic appearance of F_1 kernels with tester consisted of *su1* or *sh2* gene, the genotype at the locus responsible for kernel modification can be predicted (table 4).

Thus, the method used successfully to validate the allelic relationships among the sweet corn mutants in the present investigation can be easily translated by any maize breeder working even at remote locations without any sophisticated molecular laboratory and technical expertise. Moreover, time required for determination of allelic relationships with the method elaborated above requires only one season without any field evaluation since kernel modification or xenia effect is visible in the same generation.

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