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Modified backcross breeding method for rapid conversion of field corn line to *shrunken2* (*sh2*) gene-based sweet corn line

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Abstract

The non-availability of superior and diverse inbred parents is one of the major bottlenecks to develop high yielding sweet corn hybrids. The *sh2*, one of the promising recessive mutant alleles, has been used in sweet corn development however its transfer to superior field corn lines may require extra inbreeding to identify heterozygous carrier plants. The use of molecular markers linked with *sh2* requires a well-equipped laboratory and skilled person to carry the marker assisted backcross breeding (MABB). Considering the above constraints, a modified backcross breeding method was used taking advantage of shrunken kernel of sweet corn for foreground selection coupled with phenotypic comparison with recurrent parent (RP). The BC_1F_1 plants were grown and plants having high phenotypic similarity with RP were selfed as well as backcrossed as pollen parent. The BC_2F_1 kernels of BC_1F_1 plants having high similarity with RP and segregation in BC_1F_2 kernels were harvested. The BC_2F_1 plants phenotypically most similar to recomment parameter (RP) were selfed and the shrunken kernels were selected from the segregating BC_2F_2. The shrunken kernels were homozygous (*sh2sh2*) at *Sh2* locus, which were validated upon inbreeding based on non-segregating kernels in BC_2F_3. The modified backcross method used is rapid, cost effective and can be used by maize breeder with limited resources for conversion as well diversification of sweet corn germplasm.

Key words: Sweet corn, inbred, *Shrunken2*, foreground selection, modified backcross breeding

Introduction

Maize is a unique cereal crop having highest production and productivity worldwide. It is also unique in terms of diverse products and as an industrial crop. Sweet corn (*Zea mays* L. *saccharata*), apart from other specialty corn, namely, quality protein maize (QPM), pop corn, baby corn and waxy corn, is one of the variants of maize that is harvested and consumed at immature grain stage. Sweet corn produced and consumed prominently in many countries including, France, Thailand, Hungry, US and China. Growing sweet corn in India has occupied a prominent position among growers and consumers within a short period of time. Quality and unit production potential are the main determinant to sustain sweet corn cultivation and also to make sweet corn more competitive and remunerative crop. The availability of suitable high yielding cultivars coupled with excellent quality of sweet corn is essentially required considering both producers and consumers in India as well as in other developing countries. Although, few composite sweet corn varieties namely, Madhuri, Priya and Win-orange are available for cultivation in India (Parihar et al. 2011), however, they are poor yielder as compared to hybrids and are also having other limitations like non-uniformity associated with growing composite varieties. Recently, a few hybrids are being popularized by private sector in India but seed availability at exorbitant price makes sweet corn a difficult option for small and marginal farmers. In addition, sweet corn breeding is being intensified at public funded institutions (Mehta et al. 2017) but development of new sweet corn hybrids by public funded institutions is restricted by non-availability of superior parental lines. Further, the narrow genetic base of sweet corn may check the progress of breeding programs. The development of superior and diverse parental lines for hybrids through classical methods involving population improvement followed by inbred extraction is time taking and will not serve the immediate requirements. Although, classical backcross

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breeding can be used for transfer of a trait controlled by single gene in promising inbreds for conversion as well as diversification of germplasm, yet recessive nature of genes controlling a trait requires an inbreeding to be added for identification of heterozygous carrier individuals. Consequently, the classical backcross breeding programme requires few additional seasons for transfer of a recessive trait when compared with a trait governed by dominant gene(s) (Feng et al. 2008). The utilisation of molecular marker assisted backcross breeding (MABB) can identify heterozygous plants and thus saves inbreeding generations. The method has been used extensively for rapid transfer of traits controlled by recessive genes such as *opaque2* (*o2*) based QPM (Babu et al. 2005), β-carotene (Muthusamy et al. 2014) and for pyramiding of waxy and *opaque16*(*o16*) based QPM (Yang et al. 2013).

The MABB strategy requires polymorphic markers linked with target traits for foreground selection and polymorphic markers located across the genome for background selection (Babu et al. 2005). The involvement of molecular markers in breeding program needs a laboratory, trained human resource and additional financial support. Many traditional maize breeding centers do not have necessary infrastructure and technical manpower to exploit MABB for rapid transfer of target traits. Phenotype based foreground and background selection in backcross breeding seems to be a rapid and straight forward approach for transfer of few traits having recessive monogenic inheritance. The phenotype based foreground selection primarily requires identification of a morphological marker linked with target trait to be transferred. The trait such as sweetness in maize having xenia effect provides an advantage for utilisation of shrunken kernel character as phenotypic marker for foreground selection in a breeding programme. The xenia effect is the immediate expression of seed phenotype based on alleles present (Liu 2008), detects the presence of recessive allele in heterozygous individuals based on segregation of kernel phenotypes in ears derived through self pollination. Kernel heterozygous at shrunken locus will have normal kernel phenotype, while upon self pollination and segregation at *sh2* locus, two types of kernels will be formed on the ears. First normal kernels, due to presence of dominant homozygote and heterozygote allelic forms, second with shrunken kernels due to presence of homozygous recessive allelic form at *sh2* locus. The kernel phenotype based on xenia effect is also prominent for other alleles such as white vs. yellow kernel colour, sugary vs. non-sugary, anthocyanine *vs*. non-anthocyanine and opaque *vs*. non-opaque kernel phenotype in maize that has relevance in breeding programme. Further, maize being monoecious in nature having separate male and female reproductive parts on the same plant, it can be selfed as well as can be used as pollen parent to make backcrosses with recurrent parent (Tarcy 2001) or to make crosses with other individuals for generating seeds for progeny test. Considering the advantage of separate female and male reproductive parts in maize, and shrunken kernel associated with sweetness, we used a modified backcross breeding scheme, confirmed it to be working well, developed promising sweetcorn inbred lines and accordingly opined that this scheme is an effective approach for rapid transfer of traits controlled by recessive genes having xenia effect in otherwise elite genetic background.

Materials and methods

Plant materials

VQL373, a superior inbred line with *Sh2* allele and VSL3, a *sh2* mutant based sweet corn inbred line (Jha et al. 2016) were used in the present investigation. The VQL373 is a maize inbred line with high seed yield and good combining ability. The VQL373 was used as recurrent parent (RP) while VLS3 was used as donor parent (DP) for *sh2* allele. The materials were generated at ICAR-VPKAS Farm Hawalbagh, Almora and ICAR-DMR off-season facility at Hyderabad during 2011-2013.

Development and planting of BC_1F_1 generation

The parental lines, VQL373 and VSL3 were crossed to generate F_1. The F_1 plants were raised and backcrossed with RP, VQL373 to generate BC_1F_1. Six hundred BC_1F_1 kernels were sown in fixed manner with 2 rows of BC_1F_1 followed by 1 row of RP, VQL373. The care was taken to place the kernels at uniform depth to obtain uniform emergence of seedlings. Row length, between and within row spacing were maintained at 60 and 25 cm, respectively. The recommended package and practice were followed to raising the crop.

Selection of BC_1F_1 plants, generation of BC_1F_2 and BC_2F_1 kernels

The BC_1F_1 plants were scored for phenotypic similarity with adjacent row of RP at the flowering stage by comparison based on simple visual traits like plant height, ear placement, tassel morphology and leaf

traits and a value of 1-10 depending on extent of similarity were assigned. The plants having more similarity with RP were given high score compared to those looking different from RP. The BC_1F_1 plants scored a value of > 7 for extent of similarity with RP were selfed by supplying pollen to own silk to generate BC_1F_2 kernels. Along with selfing, the same BC_1F_1 plant were also used as pollen parent for concurrent backcrossing by supplying pollen to silk of RP plants to generate BC_2F_1 kernels.

Screening of kernel segregation in BC_1F_2 for selection of ears with BC_2F_1 kernels

The BC_1F_2 kernels derived through inbreeding were screened visually. The segregating BC_1F_2 kernels for shrunkeness on the ears of BC_1F_1 plants are supposed to be heterozygous at the *sh2* locus (*Sh2sh2*) and carrier for target gene, while those BC_1F_1 plants which did not show segregation of kernel for shrunkeness upon selfing were assumed to be homozygous dominant (Sh_2Sh_2) for the trait. The kernels from backcrossed RP ears, developed by supplying pollen from selected BC_1F_1 plants and found to be heterozygous/carrier were harvested for growing BC_2F_1 plants. From each selected ear, 2 rows of 3m length consisting of 24-25 BC_2F_1 plants were raised with recommended practice. All the plants were again scored for similarity with RP. The 5-6 desirable plants from each ear to progeny row showing high similarity (> 8) with RP were selfed to produce BC_2F_2 kernels. BC_2F_2 kernels from selfed BC_2F_1 plants which showed segregation (Fig. 2B) were shelled and only shrunken kernels were planted to raise BC_2F_2 plants. These kernels were considered homozygous recessive for *sh2* locus controlling sweetness in maize. The homozygous recessive (*sh2sh2*) BC_2F_2 plants were selfed to generate ears with BC_2F_3 kernels (Fig. 2C) to be used for further generation advancement, evaluation and utilisation in sweet corn breeding program.

Results

Generation of F_1 and BC_1F_1

The F_1 kernel between RP, VQL373 and sweet corn donor parent, VSL3 were normal kernel as expected because of allelic complementation at heterozygous shrunken locus (*Sh2sh2*). Further, all the BC_1F_1 kernels were also of non-shrunken/normal kernel type as expected because of either homozygous (*Sh2Sh2*) or heterozygous alleles (*Sh2sh2*) at shrunken locus (Fig. 1, Class I and Class II). The planting of BC_1F_1

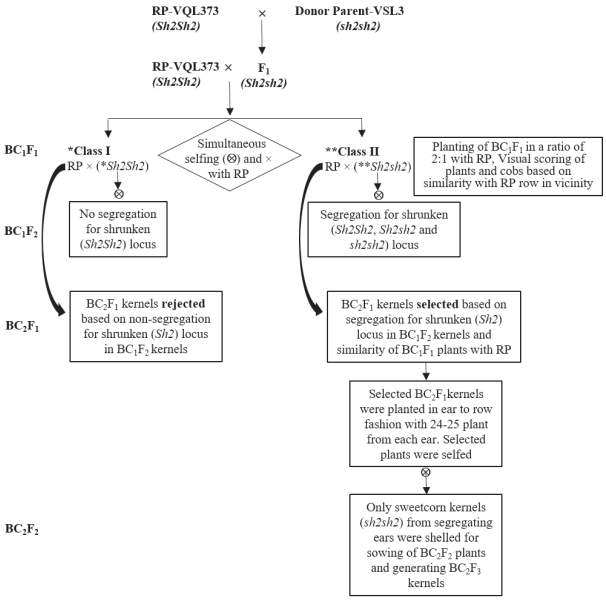

Fig. 1. Schematic representation of breeding scheme followed for selection of homozygous sweet corn plants in BC_2F_2 Generation

and RP in a ratio of 2:1 has helped to compare each BC_1F_1 plant with RP plants planted in immediate vicinity. It has minimized the environmental variability between the BC_1F_1 and RP plants and enhanced effectiveness of visual scoring for phenotypic similarity with RP.

Selection and generation advancement from BC_1F_1

Out of 600 BC_1F_1 plants grown, 108 desirable plants were selected at the flowering stage. They had more similarity to RP with visual similarity score of > 7. The selected plants were selfed to generate BC_1F_2 kernels. Concurrently each of the 108 plants were used as pollen parent and backcrossed with RP. Of the 108 selfed cobs of BC_1F_1 plants, kernels of 47 cobs exhibited segregation for shrunken and normal kernels indicating heterozygocity at *sh2* locus (*Sh2sh2*). The non-segregating selfed kernel of 61 BC_1F_1 plants indicated homozygosity at *Sh2* locus (*Sh2Sh2*) and therefore, rejected (Table 1). Fortyseven selected segregating ears were further compared with cob of RP (Fig. 2A) and based on similarity and desirable cob traits, 25 promising BC_1F_1 progenies segregating at *sh2* locus were selected. Along with selfing, 108 backcrosses were also generated using selected BC_1F_1 plants as pollen parent. Twentyfive backcrosses of the BC_1F_1 plants determined to be heterozygous at *sh2* locus and also similar to the RP for plant architecture and cob characters were selected. The

Table 1. Chi-square analysis in BC_1F_1 and BC_2F_1 generations

Backcross generation	No of plants grown	No of plants selected	No of plants plants homozygous/ non-segregating at *Sh2* locus	No of plants plants heterozygous/ segregating at *Sh2* locus	χ^2 (1homozygous: 1heterozygous)	P value
BC_1F_1	600	108	67	41	6.25	0.012
BC_2F_1	620	145	84	61	3.64	0.056

BC_2F_1 kernels of only those 25 selected plants were raised in ear to row fashion for further advancement.

Selection in BC_2F_1 and development of homozygous shrunken (sh2sh2) kernels

From each of the 25 BC_2F_1 ear to progeny row, 5-6 desirable plants were selected based on the similarity score of >8 and a total of 145 BC_2F_1 plants were selfed. Of the 5-6 selected and selfed BC_2F_1 plants, segregation in BC_2F_2 kernels at *sh2* locus was noted to vary from minimum of 1 plant to maximum of 4 plants across the 25 ear to progenies rows of BC_2F_1. The non-segregation of kernels confirmed the homozygous dominant (*Sh2Sh2*) nature of BC_2F_1 plants at the shrunken locus. The BC_2F_1 plants with kernel segregation were supposed to be heterozygous (*Sh2sh2*) at *sh2* locus. Thus, a total of 61 segregating BC_2F_1 plants were selected (Table 1). Of these, 25 promising BC_2F_1 plants, most similar with RP were retained. Only shrunken kernels (BC_2F_2), homozygous at *sh2* locus (*sh2sh2*) were shelled for the generation advancement from selfed ears of 25 selected BC_2F_1 plants (Fig. 2B). 10-12 shrunken BC_2F_2 kernels assumed to be homozygous at sh_2 locus (sh_2sh_2) from 25 segregating ears were sown to grow BC_2F_2 plants. The progeny row from each ear was evaluated for plant vigor and other desirable traits. The selfed ears of all the promising BC_2F_2 plants were consisted of only shrunken kernels (Fig. 2B). Non-segregation of kernels on BC_2F_3 ears indicated the conversion of field corn line to sweet corn line. The superior BC_2F_3 sweet corn lines selected based on desirable traits are being used as inbred parent in the sweet corn hybrid breeding program.

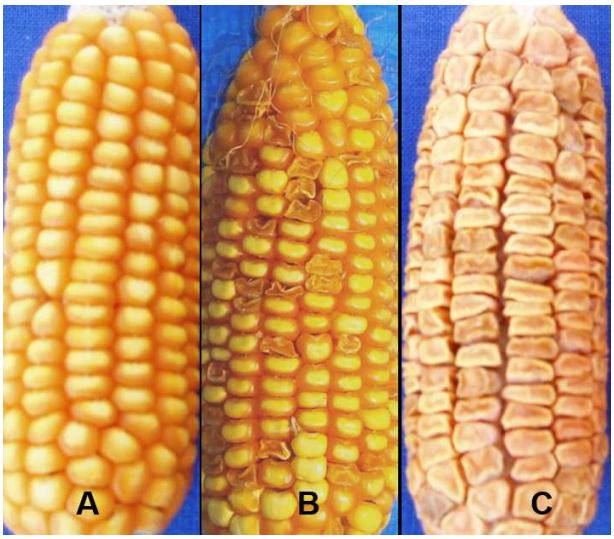

Fig. 2. A = Cob of recurrent parent, VQL373 B = Segregating sweet corn kernels in BC_2F_2 and C = Cob with BC_2F_3 Kernels on homozygous sweet corn BC_2F_2 plants

Discussion

Of the many mutant genes known in maize, *sh2* has been found to be most promising in terms of sweetness of immature maize kernels. As such it has been used in development of super sweet corn hybrids. The hybrid between *sh2* based VSL3 and *Sh2* based VQL373 and backcross progenies showed normal kernel phenotype while segregated upon inbreeding of back cross progenies. The results therefore, indicated allelic dominance of *Sh2* leading to normal kernel phenotype. The shrunken kernel phenotype is due to homozygosity at mutant *sh2* locus (Jha et al. 2016). Such an allele can be transferred in other genetic background using classical backcross breeding programme. In fact, backcross breeding has been used routinely and successfully in classical breeding programme to add a desired monogenic trait in otherwise desirable genotypes. However, the transfer of a trait governed be recessive gene(s) require more crop seasons compared to transfer of a trait governed by dominant gene(s). Molecular markers assisted back crossing technique was therefore emerged as a tool to assist breeder to select recessive allele carrier plants and make backcrossing without testing selfed progenies in ensuing seasons. The recessive quality traits namely, QPM (Babu et al. 2005) and beta-carotene (Muthusamy et al. 2014) were transferred in desirable background using MABB in maize. The

MABB is in fact a promising tool and has been integrated successfully in classical backcross breeding programme to facilitate transfer of a trait governed by recessive gene, however it needs more resources and technical expertise which may be a constraint with many maize breeding programs in the developing countries. The backcross breeding method used in the present study exploited natural phenomenon of xenia effect on endosperm as a criterion for the foreground selection to identify the heterozygous plants carrying recessive desirable allele. In fact, maize kernel colour and structure are determined by the pollen received by the ovule and used in the development of kernel and the effect is visible in the same generation without testing progenies in the next season. The plant phenotype is an indicator of its genetic content and careful observations on segregating lines and RP planted side by side may help in identification of plants phenotypically similar to RP. The selection was therefore performed by comparing the segregating plants with RP grown in vicinity of each segregating plants. The use of shrunken trait for identification of heterozygous plants, simultaneous selfing and backcrossing of segregating plants and visual selection scoring based on similarity with RP has helped in speedy conversion of the normal maize inbred line (VQL373) with *Sh2* gene in to mutant *sh2* based sweet corn lines. Further, this method has validated that the xenia effect based modified backcross breeding method can be exploited for rapid transfer of traits like sweetness controlled by recessive gene *sh2*.

The availability of molecular markers linked to the sweetness controlling genes namely *sugary1* and *shrunken2* in maize (Hossain et al. 2013) has provided the use of molecular markers for rapid conversion of non-sweetcorn line to sweetcorn lines. In marker assisted backcross breeding scheme, the foreground selection starts in BC_1F_1 plants, the plants are scored for markers linked with gene of interest and only foreground positive plants with maximum recovery of RP genome are further backcrossed. In present method used, first the BC_1F_1 plants were scored for similarity with RP and plants with phenotypic similarity score of >7 were selfed as well as backcrossed as pollen parent with RP. The foreground selection was performed subsequently based on xenia effect for kernel modification (shrunken) in segregating selfed ears in the same generation. Although, the number of plants to be selfed and further used for selection of carrier heterozygous plants is large compared with MABB, yet the cost, infrastructures and expertise involved provide an edge to the method used in present study. The planting of BC_1F_1 and RP in 2:1 has reduced the environmental variation because of soil factor and provided more precise comparison of segregating plants with RP planted in the vicinity. For conversion of normal maize into QPM lines, Babu et al. (2005) has used a BC_1F_1 population size of 178 plants and selected 3 heterozygous plants for generation advancement through 159 BC_2F_1 plants. In some other successful MABB programme at the VPKAS, a minimum of 400 plants were found to be optimum for a MABB based breeding programme. In present study, we have used a BC_1F_1 population size of 600 and selected 25 plants. The relatively large population in proposed method has increased probability to get more recombinant plants with wide range of RP contribution and therefore chance exist to identify plants phenotypically similar to RP or even better than RP. This has also given opportunity to select plant based on desirable cob traits such as tip filling and kernel rows (Fig. 2B, 2C: showing complete tip filling). The MABB method used for conversion of lines identifies the homozygous individuals in BC_2F_2 plant, although it is also possible to identify homozygous allele in BC_2F_2 kernel, which is possible by extracting DNA from seed and confirming homozygosity with linked markers. The present method exploited xenia effect in BC_2F_2 kernels to identify the homozygous shrunken kernels without any extra effort. Five seasons/generations are required for conversion of line using MABB; the same numbers of generations/seasons were taken in present method. Here, opportunity also exists to select promising superior homozygous plants from the population of homozygous individuals. Single cross sweet corn hybrids developed using converted sweet corn lines showed a significant superiority over checks in multi-location trials (data unpublished). This supports that lines developed through conversion of field corn lines as sweet corn is having potential to provide new lines in minimum period of possible time with less resources and even without a molecular laboratory. Further, this method has potential to diversify sweet corn germplasm which is essentially required to sustain sweet corn hybrid development programme.

Using xenia effect and phenotype based selection, a modified back cross method is successfully experienced and advocated for conversion of non-sweet corn line into sweet corn line. This present method does not require any molecular

breeding lab and specialized manpower and was found to be effective in terms of cost and time required.

Authors' Contribution

Conceptualization of research (SKJ, PKA, NKS); Designing of the experiments (SKJ, NKS, PKA); Contribution of experimental materials (PKA, SKJ); Execution of field/lab experiments and data collection (SKJ, PKA); Analysis of data and interpretation (SKJ, NKS, PKA); Preparation of manuscript (SKJ, NKS, PKA).

Conflict of Interest

The authors declare there to be no conflict of interest.

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