High throughput allele discovery and incorporation in elite maize germplasm

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Abstract

Genetic variation is a cornerstone of crop improvement. It serves as a tool to model crop responses to various biotic and abiotic factors. Simply stating, the germplasm with a wider base has better chance of surviving the challenges due to diseases, pests and environment. Important selection criteria such as uniformity, performance and adaptation have significantly contributed towards neglecting the superior exotic or non-elite alleles in crop species. The commercial maize breeding in the U.S. is a classical example of such efforts. Most U.S. hybrids are entirely derived from few open pollinated varieties descended from a mix of only two races. With the intention of transferring novel alleles into the mainstream maize, we selected a large number of diverse inbreds and landraces as allelic diversity donors. Two proprietary complimentary inbreds (stiff stalk and non-stiff stalk) were selected as recurrent parents to cross with the donors. Each donor is being crossed to both recurrent parents and backcrossing is continued to BC_5 generation. Over 65,000 backcross-derived near isogenic lines (NILs) distributed in 266 populations are being generated. A subset of donors is also being crossed with B73, a public maize line, and will be available for broad distribution. Each of the BC_5 populations is genotyped using an average of 140 evenly distributed SNP markers. The analysis of genotypic data showed an average of 5% donor genome with 2-3 introgressions per line. This collection of NILs enhances our ability to isolate alleles for genes of interest and test their effects in elite isogenic backgrounds.

Media summary

The collection of over 65000 near isogenic lines will enhance our ability to isolate alleles for genes of interest and test their effects in elite isogenic backgrounds. A subset of these NILs is available for broad distribution.

Key words

Genetic variation, near isogenic lines, novel alleles, maize

Introduction

The narrow germplasm base of contemporary commercial maize is well known and documented phenomenon (Goodman and Carson, 1999). Germplasm characterization studies have revealed the fact that most U.S. hybrids are entirely derived from few open pollinated varieties which were descended from a mix of only two races (Goodman and Brown, 1988; Troyer, 1999). Recycling of closely related elite inbreds for developing improved hybrids is the method of choice among commercial breeders (Troyer, 2001). This approach proves successful in the short term; however long term success of breeding programs depend upon the diversity present in the base germplasm.

The allelic variation at any locus may serve as a tool for selecting lines meeting new challenges posed by various biotic and abiotic factors. The important selection criteria such as uniformity, performance and adaptation may contribute towards neglecting exotic or non-elite maize germplasm. However, superior, novel alleles incorporated into elite, isogenic background should provide useful and immediate tools for improvement of existing commercial maize cultivars. With the objective of developing this tool for Syngenta maize breeders, we are developing a large collection of backcross derived inbred lines (BILs). A diverse set of inbreds and landraces is being used as allelic diversity donors to cross with two proprietary complimentary inbreds. A small subset of inbreds is also being crossed with a public line and will be available for distribution*.

*public access of the B73 materials is subject to limitations on IP establishment on derived materials and information. Also publication of results enabled by this material is encouraged.

Methods

Characterization of allelic diversity donors

The initial list of 300 potential allelic diversity donors was narrowed down to 134 based on the genotypic data using SSRs and gene sequences. Sixty-five diverse inbreds and 69 individual representative of landraces were selected based on molecular distances (unpublished data provided by John Doebley) as well as classification of races of corn by Goodman and Brown (1988) for platform development. Further genotypic, geographic and phenotypic characterization of this set is underway.
The seed for landraces and public inbreds was provided by Mark Millard (USDA-ARS, North Central Regional Plant Introduction Station, Ames, Iowa). One hundred and twelve out of 134 donors were genotyped with 800 SNP markers. Geographic and phenotypic characterization of the allelic diversity donors includes gathering information on ear, plant characteristics, their geographic origin, and breeding value (if any) is being collected.

Population development

A total of 293 populations of backcross derived inbred lines are being developed by crossing 134 allelic diversity donors with two complimentary proprietary inbreds. A small subset (25 out of 65) of diverse inbreds is also being crossed with a public line, B73, and will be available for distribution. Backcrossing will be continued until BC5 generation. Each population is comprised of 250-350 BC5 families. Total of 197 populations are generated so far.

Genome-wide genotypic scan of populations

A total of 230-350 BC5 families per population were grown in the greenhouse and tissue samples were collected for eight plants per family and bulked. Each population was genotyped with 128-150 evenly distributed SNP markers. Out of 197 populations generated so far, 145 were genotyped and analyzed. The results of analysis of genotypic data for some of the representative populations are presented here.

Results

Characterization of allelic diversity donors

The genetic diversity among the donors in the platform was observed to be of larger magnitude as compared to the Syngenta proprietary inbreds and diverse inbreds used in the public domain (Figure 1).

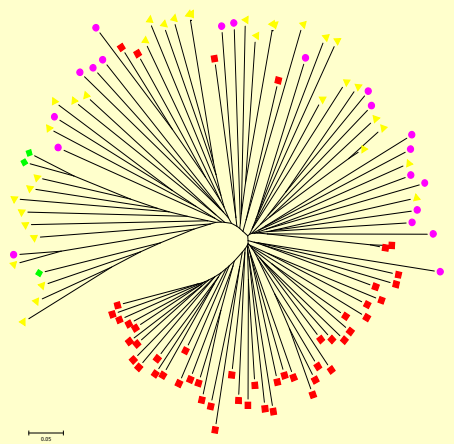

Fig. 1 Phylogenetic tree constructed using 800SNP markers for 112 allelic diversity donors, a. 25 diverse inbreds (●) crossed with B73, b. Landraces (■), c. proprietary inbreds (▲), d. recurrent parents (◆)

The genotypes from 21 different countries including USA are represented in the platform (Figure 2).

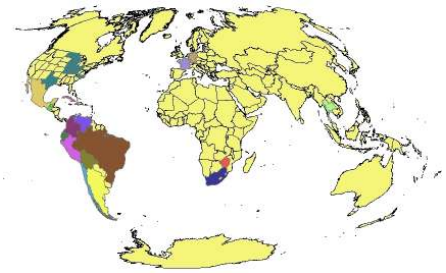

Fig. 2 Geographic distribution of allelic diversity donors

Genome-wide genotypic scan of populations

The donor genome content in the populations analyzed so far ranged from 5.0 to 7.0% and the recurrent parent genome content ranged from 91.3 to 93.4% per family (Figure 3). The average

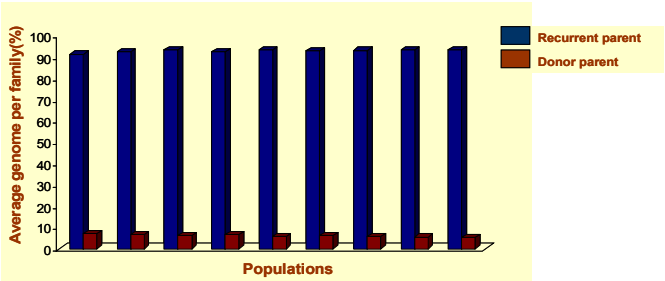

Fig. 3 Distribution of genomic constitution of nine populations in terms of donor and recurrent parent genome percentage

number of introgressions ranged from 3.15 to 4.9 and the average size of introgression ranged from 18.5 to 33.09cM per family (Figure 4). The donor genome was equally distributed on all the

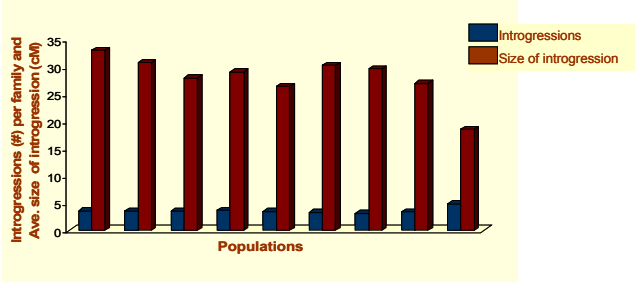

Fig. 4 Distribution of genomic constitution of nine populations in terms of average number of introgressions and average size of introgression

linkage groups for populations analyzed so far. An example is shown in figure 5. The amount of donor genome ranged from 4.9 to 7.0% per linkage group. Further analysis was done to reduce

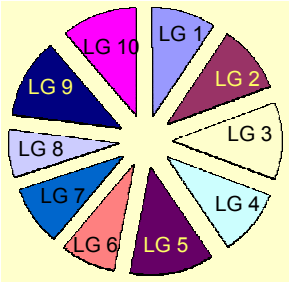

Fig. 5 Distribution of donor genome on individual linkage groups of a population

the number of families covering entire genome carrying minimum number of introgressions and smallest size of individual introgression. The number for several populations analyzed so far ranged from 76 to 95. A representative picture of a population is shown in figure 6.

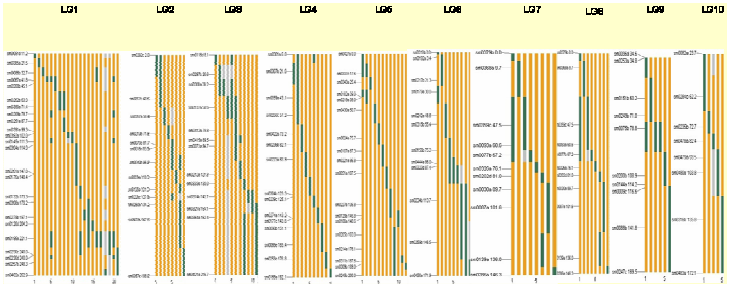

Fig. 6 The distribution of donor genome introgressions covering all linkage groups of a population

Conclusion

This large collection of NILs will be used for various forward and reverse genetics approaches. It enhances our ability to isolate alleles for genes of interest and test their effects in elite isogenic backgrounds.

References

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Syngenta will abide by all applicable biodiversity laws in our use of exotic germplasm