Maize mutants a boon to society in past and in future what…?

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Abstract

Any altered plant phenotypic expression is a variation that may be desirable or undesirable. Mutants form one such class which has been exploited in past and have role to play in future. One of the aspects in utilizing the worth of mutants is conversion of normal maize into quality protein maize (QPM). In the current investigation we have found a number of plants in the BC_2F_1 populations whose phenotypic expression were different from normal plants. The most susceptible population was $BC_2F_1/11$ where frequencies of altered phenotypes were maximum (42.42 %) whereas minimum of 2.08 % plants were noted with altered phenotypes in $BC_2F_1/2$. Further, altered plant phenotypes were grouped according to their similarity to identify the severe type phenotypic alteration. Based on grouping, cob at top without tassel was identified to be the most frequent one with 25 plants showing such types of phenotypic alteration. The unusual phenotypes found minimum was one each in earless, tasseless with cob cluster, branched and cob and tassel together at top mutants.

Introduction

Maize is a model biological system as well as an important agronomic crop. While it is anticipated that novel mutants that enhance our understanding of maize as a biological organism will lead to applications that improve this plant as a crop, it is also clear that some information from basic maize mutant research can have direct applications. One classic example is the discovery that mature *shrunken2* (*sh2*) mutant kernels are sweet (Laughnan, 1953). This led to a revolutionary change in the sweet corn industry, the transition from the traditional sugary varieties to the *sh2*-based super-sweet varieties (Tracy, 1997). This turned fresh sweet corn from a local seasonal crop to one that can be enjoyed year round. Laughnan (1953) originally used the *sh2* mutant because of its tight genetic linkage to the anthocyanin pigment synthesis factor, *a1*, an early example of marker-assisted selection. More recently, it was discovered that *sh2* encodes the 60 kDa subunit of endosperm ADP glucose pyrophosphorylase, an enzyme essential for starch synthesis (Bhave *et al.,* 1990). Other mutants such as *sugary1, sugary enhancer1, opaque2, floury2, waxy1, amylose extender1, Leafy1*, and *brown midrib3*, have also been used in direct applications for specialty corn production (Cox and Cherney, 2001; Hallauer, 2001).

In maize, the shoot apical meristem (SAM) typically initiates a fixed number of leaves during the vegetative phase of growth. The initiation of many leaves is accompanied by the formation of a new meristem in its axil called the axillary meristem. The shift from vegetative to reproductive development is a significant transition in the maize life cycle. During this transition, the SAM ceases to make leaves and transforms into an inflorescence meristem, which subsequently produces a number of specialized lateral meristems that ultimately lead to the formation of the tassel (male inflorescence) (Russell and Stuber, 1983; Irish and Nelson, 1991). The tassel is a branched inflorescence consisting of one central spike and several basal branches that together bear the spikelets and florets containing the floral organs. To initiate tassel formation, the inflorescence meristem gives rise to several files of branch meristems spanning its entire length. These branch meristems either elongate to form long lateral branches or become spikelet pair primordia, which branch again to form two spikelet meristems. Each spikelet meristem goes on to initiate a pair of floral meristems that give rise to the floral organs (Kiesselbach, 1949; Cheng *et al.,* 1983). In contrast to the tassel, ears (female inflorescences) develop from one or more axillary meristems on the main axis of the plant. The ear develops as a single thick rachis without basal branches (Kiesselbach, 1949; Veit *et al.,* 1993; McSteen *et al.,* 2000). There exists in maize several curious mutants where one or the other inflorescence fails to form altogether. The *tasselless1* (*tl1*) mutants lack a tassel at maturity, but can produce ears and may otherwise appear normal. Anecdotal evidence suggests that this is a phototropic response that can be ameliorated by growth under short days, but detailed studies on this mutant are lacking. Similarly, the mutants *barren stalk2* and *3* lack ears and tillers but produce an otherwise normal plant including a normal tassel (Pan and Peterson, 1992; Neuffer *et al.,* 1997). In the case of *ba2*, at least, ears shoots are initiated but then arrest their development at such an early stage that the shoot never emerges from the axil of the leaf. Unlike *ba1*, which affects initiation of all axillary meristems, *ba2* and *ba3* are specific to the axillary shoot meristem, but affect the continued growth of the shoot rather than its initiation or formation. The recessive andromonoecious dwarfs fail to synthesize normal levels of gibberellic acid (GA) and normal stature and floret development can be rescued by GA application (Phinney, 1956; Phinney and West, 1960). The group of tasselseed mutants shares in common the partial to nearly complete conversion of the normally male tassel spikelets into ones that bear female florets

(Nickerson and Dale, 1955). This often results in seeds being formed on the tassel, sometimes as profusely as on the ear. Two groups of tasselseeds were established based on genetic analyses and morphological considerations with *ts1*, *ts2*, *Ts3*, and *Ts5* falling in the group where sex reversal was not accompanied by extra branching, and *ts4* and *Ts6* affecting sex determination and causing proliferative inflorescence branching (Irish *et al.,* 1994). The *ts2* was the first tasselseed gene to be cloned (DeLong *et al.,* 1993). It encodes a short chain alcohol dehydrogenase that is expressed in both ear and tassel spikelets. This leads to an apoptotic-based degeneration of the gynoecium in the tassel florets as well as in the lower floret of the ear (Calderon-Urrea and Dellaporta, 1999).The *silky1* (*si1*) mutant which produces numerous silks emerging from both tassel and ear spikelets. A mutant tassel producing silks is reminiscent of the tasselseed class of mutants. However, in the case of *si1* mutants the silks arise in place of stamens due to homeotic floral organ conversions. The *si1* encodes a MADS-box gene related to the B-class floral homeotic MADS-box genes *APETALA3* of *Arabidopsis* and *DEFICIENS* of *Antirrhinum* (Ambrose *et al.,* 2000).

The discoveries that arise from research on maize mutants will undoubtedly enhance our understanding of maize as a biological organism. Investigation on diverse allelic variants will probably lead to other revolutionary enhancements that can be applied to maize and other crop plants to help feed humanity in the future. Considering the significance of allelic variants in maize improvement, we characterised the various types of altered plant phenotypes appeared in BC_2F_1 population, developed for conversion of normal maize into quality protein maize (QPM).

Materials and methods

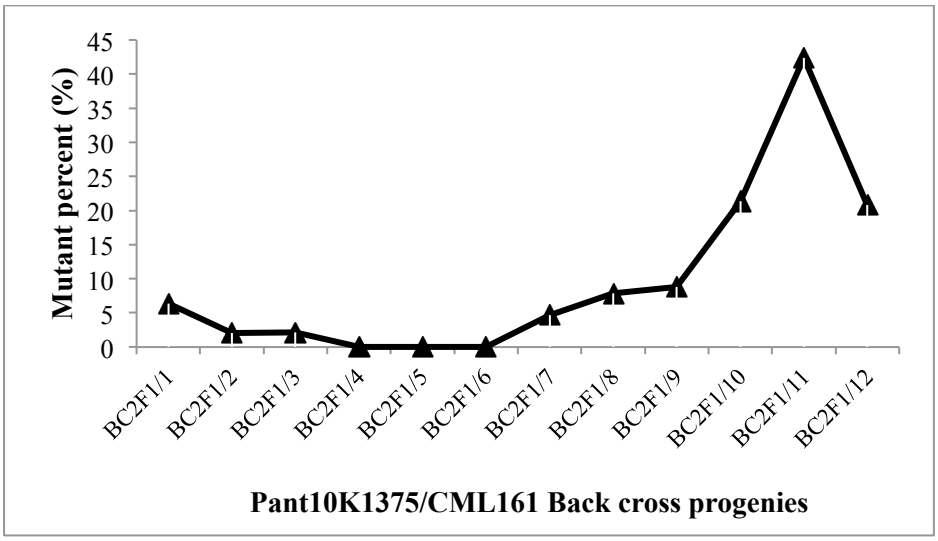
The materials comprised of BC_2F_1 populations derived from crosses between Pant 10k1375 x CML161 and backcrossed with recurrent parent (Pant 10k1375). The Pant10K1375, a normal maize inbred line, developed in Maize Breeding Programme at Pantnagar. The CIMMYT Maize Line 161 (CML-161), a QPM inbred lined, was developed by CIMMYT. The F_1 of initial cross were backcrossed with recurrent parent, and BC_1F_1 and BC_2F_1 plants were selected for heterozygous *opaque2* allele (o2) using SSR markers for conversion of normal maize line (Pant 10k1375) into QPM. Progeny populations of all the 12 plants found positive based on the *phi057 and umc1066* SSR markers for *o2* allele were planted on 09.09.2012. The selected plants were self pollinated to generate the BC_2F_2 populations for conversion programme. Out of the progenies population of 12 plants, initially selected for heterozygous *o2* locus, some were found to have altered phenotypic expression than the normal type. The phenotypic differences were related to plant height, position of ear placement, differential expression of reproductive organs. Such kind of changes were across the progeny populations of 12 plants characterized for plant height, number of ears, effective ears, barren ears, branching pattern.

Results and discussion

A global climatic change is now considered to be underway and is expected to result in a long term trend towards changes in environmental conditions. Congenial environmental seasons support optimal development, however, unfavourable environments influence the genetic architecture of the plant and reduce yield directly by affecting plant growth and development, and indirectly by modifying the normal plant phenotype. On the other hand, altered plant phenotype may serve as valuable source of variability for improvement of specific trait or even can help in development of specific purpose corn as happened in past in development of specialty corn such as sweet corn, QPM etc. Unpredictability of weather conditions has occasionally resulted in many unusual expressions in plant characteristics in general, and ear and tassel characteristics in particular, in maize. Multiple ears on single nodes are one of the environmentally induced oddities widely reported in maize. The expression of multiple ears in inbred lines, populations and experimental hybrids was also recorded in maize grown in the Tarai region of Uttarakhand, India (Singh *et al*., 2009; Singh and Devi, 2010). The twin ear expression on 33 single nodes in maize was observed earlier by Hallauer in 1973 in S2 and S5 progenies of two populations (Hallauer, 1984). The unusual expressions were observed in BC_2F_1 population planted during September and flowered during early November 2012. The temperature during the sowing and initial vegetative growth was normal whereas during pre-flowering, flowering, post-flowering and grain filling stages, the temperature was lower than the normal required for maize. The unusual expressions include the expression of silks in tassel, part of the tassel converted into an ear, plants with terminal ears without any tassels, earless and terminal as well basal branching. In fact, unisexuality in maize occurs through the selective elimination of stamens in ear florets and by elimination of pistils in tassel florets. The two general classes of sex determining mutants have been identified in maize, including those of masculinized ears and feminized tassels. The endogenous gibberellic acid (GA) has been found to have a feminizing role in sex determination in maize (Tanurdzic and Banks, 2004). Moreover, reversal of sexual expression in maize has been shown to be influenced by environment and heredity (Richey and Sprague, 1932; Heslop-Harrison, 2008). The altered phenotypic expressions observed in the present investigation have been described on ensuing pages.

The observations indicate that progenies of $BC_2F_1/4$, $BC_2F_1/5$, $BC_2F_1/6$ did exhibit plants with altered plant phenotypes. The most susceptible population was $BC_2F_1/11$ where percentage of mutant phenotypes was maximum (42.42 %) whereas minimum percentage of 2.08 % was found of $BC_2F_1/2$ altered phenotypes (Fig.1). Further, altered plant phenotypes were grouped according to their similarity, to identify the severe type phenotypic alteration. Based on grouping, cob at top without tassel was identified to be the most frequent one with 25 plants showing such types of phenotypic alteration. The unusual phenotypes found minimum was one each in earless, tasseless with cob cluster, branched and cob and tassel together at top mutants (Fig.2).

Fig.1: Frequency of mutants in Backcross progenies in maize



Dwarf mutant

The heights of these mutants were in the range of 29-77 cm and had presence of tassel and ear. The ears having normal silk but the tassel was small in the form of cluster (abnormal). Seed development was observed in these cobs since other plants having normal tassel development were present in the vicinity of the plant. Intermodal distance between the nodes is minimized with less leaves. This may be due to suboptimal synthesis of endogenous gibberellic acid (Fig.3a).

Fig.3a Dwarf mutant

Fig.3b Cob and tassel together at top

Tassel and cob together at top

Tassels with both anthers and ears. Some plants were found to have both sexual expressions in the tassel. Generally, the main rachis of the tassel was converted into a small ear that set seed, whereas the remaining tassel branches developed anthers with pollen grains along with few seeds on tassel spikelets (Fig.3b). This type of mutant showed similarity with tasselseed mutants (Nickerson and Dale 1955).

Cob at top without tassel

Maize plants normally consist of terminal tassels as male inflorescences and lateral ears as female inflorescences. In 25 plants, however, the terminal tassels were entirely modified into small single ear with silk in place of tassels (Fig.3c). These ears were found to have seed set. However, the size of the cob and number of grain varied from plant to plant and also smaller than the size and number on normal ear.

Fig.3c Cob at top without tassel

Fig.3d Earless

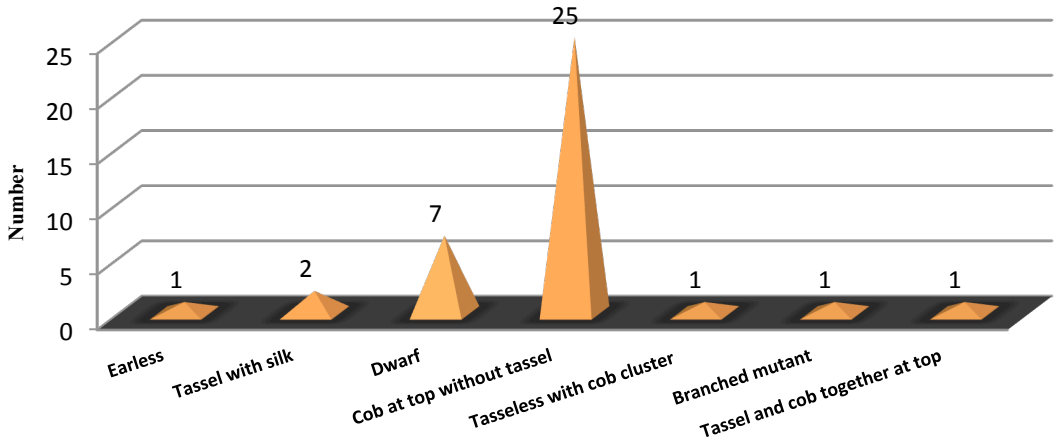


Fig. 2: Frequency of Mutant Types

Earless mutant *(Barren stalk 1)*:

Inflorescence mutant of maize named *barren stalk1 (ba1). barren stalk1* is a recessive mutant of maize that has no tassel branches, spikelets, tillers, or ears, first identified in 1928 (Hofmeyer, 1930). In the original report, the *ba1* mutant was described as having defective tassel branching and a central stalk devoid of any ears. *ba1* mutants are disrupted in the initiation of both vegetative and inflorescence axillary meristems. This defect manifests itself as a failure to produce vegetative branches (tillers), branches in the tassel, spikelets on the tassel's central spike, and ears. In the present investigation, we found one plant having no ear but normal tassel in BC_2F_1-1 plant population (Fig.3d).

Branched mutant

Occurrence of lateral and basal branching was observed in this type of mutant, terminating with a cob either singly or in clusters. Height of these plants was lower than the normal and completely lacked the male reproductive part tassel (Fig. 3e).

Fig.3e Branched

Fig.3f Tassel with silk

Fig.3g Tasseless with cob cluster

Tassel with silk

The tassel is the terminal male organ, consisting of anthers and producing pollen grains for fertilization of the ovule, which is borne in the so-called lateral ear. In two such plants, rudimentary tassel with profuse silk without ear was observed. Such plants were also of short height with less intermodal distance (Fig. 3f).

Tasseless with cob in cluster

A single plant was found to have many ears on separate nodes without tassel formation. In such cases, ears were lacking silks and with extended leafy sheath. As a result, pollination could not take place and ears remained barren or set very few seeds. Such plants were also branched laterally and also less in height than the normal plants (Fig. 3g).

The unusual expressions in ear, tassel, plant height and branching may be due to environmental factors as the pre-flowering, flowering, post-flowering and grain filling stages are coincide with the low temperature along with short day length. There is also probability of involvement of genetic factors or interaction of genetic and environmental factors. However, progeny analysis or other genetical studies are required to be carried out to confirm the basis of plant phneotypes alteration. Such analysis is also essential to assess the worth of such phenotypically modified plants in maize improvement programme. Richey and Sprague (1932) reported the role of environment, *i.e.,* shorter daylight periods and lower temperatures, and heredity in the development of silks in the tassels. Heslop-Harrison (2008) also shared the viewpoint that low temperatures, particularly when experienced through the dark period of the daily photoperiodic cycle, promote female sexual expression and depress male. In case of widespread occurrence of unusual plant phenotypes, the quality as well as the quantity of the maize grain or green cob will certainly suffer.

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