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Enhancement of Maize Allelic Diversity using Wild Relative Teosinte (*Zea mays* ssp. *Parviglumis*)

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Teosinte (*Zea mays ssp. Parviglumis*), a wild relative of maize was assumed to be a potential donor for novel gene(s) for diversification and enhancement of maize germplasm. Subsequently teosinte was crossed with three maize inbred lines Tarun 83, DMRHyd 1284 and Pant 12K 53 followed by one generation of back crossing and two generation of selfing. The teosinte derived BC_1F_3 lines from all the three crosses were evaluated during spring season 2016. Analysis of variance revealed significant variance for most of the characters in all the three teosinte derived maize lines. Variability parameters revealed low variations for maturity traits namely days to 50% anthesis and silk emergence, days to 50% leaf senescence and anthesis-silking interval. Plant height exhibited wide range of variation and in general plant height of teosinte derived lines was more than the plant height of maize lines. Grain yield/plot was the most responsive trait because of the maximum variance observed in teosinte derived lines. Other traits exhibiting fairly high diversification in teosinte derived lines were cobs/plot followed by tassel branches and flag leaf length. The results, therefore, indicate that teosinte can be used effectively for domestication of wild alleles and diversification of maize germplasm. In the present investigation, only few morphological traits were analysed, however, teosinte can also be used for diversification of abiotic and biotic stress tolerance.

Key Words: Allelic diversity, Maize, Teosinte, Wild Relative

Introduction

Maize (*Zea mays* ssp. *mays*) is an important cereal worldwide, used primarily as food, feed and to create a variety of food and non-food products, such as corn flour, corn meal, sweeteners, corn oil, starch and ethanol. Racial diversity has always been the basis for productivity enhancement to support continuous increasing demand of maize. However, selective breeding amongst few promising has accelerated genetic erosion, a phenomenon that is obvious globally as well as locally. Despite being one of the species with greater genetic diversity, molecular analysis of the maize genome suggests that a single domestication event reduced diversity when compared with wild relative (Vigouroux *et al*., 2002; Warburton *et al*., 2008). Most maize commercial varieties in the world has limited genetic diversity, whereas, today the germplasm base in maize breeding programs is relatively narrow (Tarter *et al*., 2004; Le Clere *et al*., 2005; Liu *et al*., 2016). Domestication and breeding bottlenecks have resulted in genome-wide reductions in genetic variation in maize relative to teosinte (Tenaillon *et al*., 2004). Additional studies indicated that approximately 2-4% of genes were targets for artificial selection during domestication and breeding (Wright *et al*., 2005; Hufford *et al*., 2012), which implies that about 500 to 1,000 genes were critical during the evolution of modern maize, and are prime subjects for evolutionary and agronomic research. Large differences in plant morphology between maize and teosinte make phenotypic comparisons difficult. Photoperiod sensitivity, ear morphology, and kernel traits are among the most distinguishing characters between maize and teosinte (Iltis, 2000). In spite of larger genetic polymorphism in teosinte, limited efforts have been made to generate the genetic resources and tap the allelic diversity of teosinte for diversification and maize germplasm enhancement (Liu *et al.,* 2016). To enhance and broaden genetic base of maize germplasm, there is need to explore potential of teosinte in maize breeding programme. With elementary initial knowledge, the present investigation was planned to utilize teosinte in crossing programme for domesticating wild alleles for enhancing genetic diversity in maize.

Materials and Methods

With initial observations on teosinte (*Zea mays ssp. Parviglumis*), the present investigation was planned to diversify maize inbred lines through teosinte hybridization. Consequently, three inbred lines of maize namely Tarun ⊗ 83-1-3-2-⊗-1-2-1 (Tarun83), DMR Hyd

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–1284-⊗-1-1-1 (DMRHyd 1284) and Pant 12K/NAIP II/53⊗-⊗-1-1-1 (Pant12K53) were selected and raised in crossing block of Maize Breeding plots during *kharif* 2013 at Norman E. Borlaug, Crop Research Centre of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. India. Teosinte was also sown in the same field 20-25 days earlier than the maize sowing. Maize inbred lines were used as seed parent and at the time of silk emergence hand pollination was made by collecting pollen in tassel bag from teosinte. The F_1s were backcrossed followed by self-pollination to generate BC_1F_2 and BC_1F_3 populations in subsequent years. Since the purpose of this programme was to develop improved inbred lines, therefore selection during the back crossing and selfing was exercised. In total 88 teosinte derived maize lines, comprised of 40 BC_1F_3 lines of Tarun 83, 24 BC_1F_3 lines of each of the DMRHyd 1284 and Pant12K 53 along with three parental lines, were evaluated during *Spring* 2016 in a Randomized Block Design replicated thrice. Each line was sown in 3 m row spaced 75 cm and plant to plant distance was kept to be 20 cm. Irrigations were stopped at pre-flowering stage and allowed the crop to grow naturally under existing conditions of low moisture and high temperature. Observations were recorded on days to 50% anthesis, days to 50% silking, anthesis silking interval (ASI), days to 75% leaf senescence, plant height, tassel length, number of branches/ tassel, flag leaf length (cm), flag leaf width (cm), number of cobs/plot and grain yield/plot. Traits related to fodder i.e. basal branching, plant height, leaf length and width, biomass production per plant were recorded. Data were analyzed using Windostat to determine statistical significance.

Results and Discussion

Analysis of variance revealed significant variance for all the characters in all the three teosinte derived maize lines except for ASI and flag leaf width in teosinte derived lines of DMRHyd 1284 (Table 1). Significant variance, therefore, indicates reshuffling of maize genome due to introgression and recombination of alleles from teosinte. Teosinte derived maize lines were observed to be phenotypically different in terms of more height, tassel shape and size, and many plants bears multiple ear. Silk colour and purple tinge on glume, the two prominent characters of teosinte, were observed in most of the teosinte derived lines of all the three crosses. Thus, morphological variation amongst the teosinte derived lines indicates wide range of allelic variation in the maize genome.

Days taken to 50% anthesis varied from 71.0 to 86.7 days with general mean of 73.9 days and mean of Tarun 83 of 82 days. The PCV and GCV for anthesis duration were 5.3% and 4.5% (Table 2). Minimum days required for silk emergence was 71.0 whereas it was maximum of 89.7 days in teosinte derive maize lines whereas the maize line Tarun 83 exhibited 50% silk emergence in 85.7 days. The overall mean for days to silk emergence was 75.9 days with PCV and GCV of 5.8% and 4.5%, respectively. Anthesis-silking interval (ASI) for Tarun 83 was 3.7 days with overall mean of 2.0 days. ASI varied from 0.7 day to 4.0 days with PCV of 65.6% and GCV of 27.8%. The overall mean for days to senescence was 114.5 whereas Tarun 83 took 117 days in senescence. Minimum and maximum days to senescence were 109.0 and 119.0 days with PCV and GCV of 2.4% and 2.1%, respectively. Twenty five BC_1F_3

Table 1. Analysis of variance for different characters in teosinte derived maize lines

S. No.	Character	Mean square (MS)		
		Tarun 83	DMRHyd 1284	Pant12K 53
1.	Days to 50 % anthesis	37.8**	31.3**	69.2**
2.	Days to 50% silk emergence	46.5**	41.9**	123.1**
3.	Anthesis - Silking Interval (ASI)	2.4*	1.9	12.5**
4.	Days to 75% leaf senescence	19.9**	11.1**	22.2**
5.	Plant height (cm)	895.6**	1198.8**	2525.4**
6.	Tassel length (cm)	30.7**	49.3**	17.8*
7.	Branches/tassel	44.9**	50.9**	33.8**
8.	Flag leaf length (cm)	12.1**	27.1**	70.1**
9.	Flag leaf width (cm)	0.6**	0.4	0.9**
10	Cobs/plot	49.5**	17.9**	35.7**
11.	Grain yield/ plot (g)	99298.5**	82160.0**	114078.0**

*, **-denotes 5% and 1% level of probability, respectively.

Table 2. Genetic variability parameters for different traits of teosinte derived maize lines of Tarun 83

S. No.	Character	Mean		Range	PCV (%)	GCV (%)
		Tarun 83	General mean			
1.	Days to 50% anthesis	82.0	73.9	71.0-86.7	5.3	4.5
2.	Days to 50% silking	85.7	75.9	71.0-89.7	5.8	4.9
3.	Anthesis - Silking Interval (ASI)	3.7	2.0	0.7-4.0	65.6	27.8
4.	Days to 75% leaf senescence	117.0	114.5	109.0-119.0	2.4	2.1
5.	Plant height (cm)	105.0	131.6	105.0- 171.7	14.1	12.6
6.	Tassel length (cm)	24.0	28.6	21.5- 36.1	12.7	10.3
7.	Branches/tassel	14.1	18.0	11.1- 26.9	23.4	20.5
8.	Flag leaf length (cm)	25.6	25.8	16.5- 35.9	16.8	12.2
9.	Flag leaf width (cm)	3.9	3.7	2.9- 4.9	9.64	4.7
10.	Cobs/plot	4.7	9.3	2.3-18.0	45.2	42.9
11.	Grain yield/ plot (g)	120.0	309.3	80.0-820.0	58.9	58.7

lines (including maize line) evaluated from each of the other two crosses namely DMRHyd 1284 and Pant12K 53 had by and large similar pattern of observations for days to anthesis, days to silk emergence, ASI and days to senescence as noted for BC_1F_3 lines derived from crossing Tarun 83 with teosinte (Table 3, 4). The PCV and GCV for days to anthesis, days to silk emergence and days to senescence are quite low yet results indicate diversification of maize lines due to wide range of observed variation. Some of the teosinte derived maize lines are significantly lower in maturity traits while some others are significantly higher than the maize parental lines. Low variability in maturity traits is inevitable since selection was exercised for relatively uniform maturity during backcrossing and selfing. ASI is one of the important parameter that determines productivity under normal as well under stress environment. Minimum ASI (<4 days) ensures complete pollination and seed setting whereas frequency of partial seed setting or even no seed setting at all increases with increasing ASI. In the tesosinte derived lines, ASI was normal or within the limit except in teosinte derived maize lines of Pant12K 53 where general mean for ASI was 3.1 days and range was varied from 3.1 to 9.0 days. Observations on ASI across the three teosinte derived maize populations indicates Pant12K 53 as relatively more sensitive to environmental stress as indicated by 8 days differences in days to anthesis and days to silk emergence.

Leaf senescence is another physiology related parameter that is associated with terminal drought or heat stress. Parental mean, general mean, range, PCV, GCV for days to 75% leaf senescence were 117.0, 114.5, 109.0-119.0, 2.4%, 2.1% in Tarun 83, 116.0, 116.3, 113.0-119.3, 2.0, 1.5 in DMR Hyd 1284 and 118.0, 117.7, 108.7-123.0, 2.6%, 21% in Pant12K 53-teosinte lines, respectively.

Plant height is one of the traits that have drastic effect when maize and teosinte is crossed. Tarun 83 maize line had plant height of 105.0 cm whereas in Tarun 83-teosinte lines, plant height varied from 105.0 to 171.7 cm with general mean, PCV and GCV of 131.5 cm, 14.1% and 12.6%, respectively. Plant height of DMRHyd 1284 was 69.7 cm whereas its derived lines varied in plant height from 69.7 to 174.3 cm with mean of 125.2 cm. PCV and GCV for plant height were 17.0% and 15.5%,

Table 3. Genetic variability parameters for different traits of teosinte derived maize lines of DMRHyd 1284

S. No.	Character	Mean		Range	PCV (%)	GCV (%)
		DMRHyd 1284	General mean			
1.	Days to 50 % anthesis	86.3	74.1	70.3-86.3	5.1	3.9
2.	Days to 50% silk emergence	89.3	75.5	70.7-89.3	5.6	4.6
3.	Anthesis - Silking Interval (ASI)	3.0	1.6	0.3-3.3	79.1	29.2
4.	Days to 75% leaf senescence	116.0	116.3	113.0-119.3	2.0	1.5
5.	Plant height (cm)	69.7	125.2	69.7-174.3	17.0	15.5
6.	Tassel length (cm)	13.8	27.6	13.9-36.6	16.0	14.0
7.	Branches/tassel	10.3	20.7	10.3-30.3	22.0	18.7
8.	Flag leaf length (cm)	25.5	29.5	24.9-34.3	13.6	7.9
9.	Flag leaf width (cm)	3.1	3.8	3.1-4.7	15.0	4.5
10.	Cobs/plot	5.7	9.6	5.7-14.7	28.1	24.0
11.	Grain yield/ plot (g)	69.7	371.4	69.7-680.0	44.6	44.5

Table 4. Genetic variability parameters for different traits of teosinte derived maize lines of Pant12K 53

S. No.	Character	Mean		Range	PCV (%)	GCV (%)
		Pant12K 53	General mean			
1.	Days to 50 % anthesis	86.0	77.7	71.3-87.0	6.6	6.0
2.	Days to 50% silk emergence	94.7	80.8	72.7-94.7	8.3	7.7
3.	Anthesis - Silking Interval (ASI)	8.33	3.1	3.1-9.0	72.5	62.92
4.	Days to 75% leaf senescence	118.0	117.7	108.7-123.0	2.6	2.1
5.	Plant height (cm)	98.0	120.2	48.0-158.7	26.1	23.1
6.	Tassel length (cm)	24.4	26.6	21.0-29.7	13.2	6.2
7.	Branches/tassel	18.6	18.1	10.3-23.9	23.7	15.3
8.	Flag leaf length (cm)	17.0	25.7	15.9-33.8	21.9	17.0
9.	Flag leaf width (cm)	3.4	3.5	2.6-4.7	18.5	13.1
10.	Cobs/plot	1.3	6.8	1.3-15.7	53.7	48.8
11.	Grain yield/ plot (g)	15.0	269.4	15.0-670.0	72.5	72.3

respectively. In case of Pant12K 53-teosinte lines, plant height varied from 108.7 to123.0 cm with general mean of 118.0 cm and mean plant height of Pant12K 53 of 117.7 cm. Variations in plant height in teosinte derived maize lines along with PCV and GCV observed in the investigation clearly indicates the diversification in plant height when compared with the height of maize line. In addition, teosinte derived maize lines have become sturdier than the maize plant.

Tassel length and branching are also influenced under stress environment and in the investigation variable response has been observed. In all the three teosinte derived maize populations, tassel length and tassel branches showed wide range of variations along with moderately high PCV and GCV. General mean for tassel length and range of variations were 28.6 cm, 21.5- 36.1 cm, and 27.6, 13.9-36.6 cm, and 26.6, 21.0-29.7 cm in teosinte derived maize lines of Tarun 83, DMRHyd 1284 and Pant12K 53. Number of branches/tassel in teosinte derived lines from Tarun 83 varied from 11.1- 26.9 with parental mean and general mean of 14.1 and 18.0. DMRHyd 1284 possessed tassel branches of 10.3 whereas its derived lines varied in tassel branching from 10.3 to 30.3 with general mean of 20.7. Teosinte derived lines of Pant12K 53 had average of 18.1 branches each tassel whereas minimum and maximum number of tassel branches were noted to be 10.3 and 23.9. Tassel branches in Pant12K 53 were 18.6.

Flag leaf length and width are again physiologically important parameters. Both the parameters exhibited variation in teosinte derived maize lines. Tarun 83 had flag leaf length of 25.6 cm whereas its derived lines varied in flag leaf length from 16.5 to 35.9 cm with overall mean of 25.8 cm. PCV and GCV for flag leaf length were 16.8% and 12.2%, respectively. Parental mean, general mean, range, PCV and GCV for flag leaf length in DMRHyd 1284 and its derived lines were 25.5 cm, 29.5 cm, 24.9-34.3 cm, 13.6% and 7.9%, respectively. Pant12K 53-teosinte lines possessed flag leaf length from 15.9 to 33.8 cm with mean of 25.7 cm while parental line Pant12K 53 had flag leaf length of 17 cm. The PCV and GCV were 21.9% and 17.0% in teosinte derived lines of Pant12K 53.

Number of cobs/plot depends on the plant ability to bear one or many cobs. Effective cobs/plot reduced under abiotic stresses including high plant density. During the stress, many plants remain barren either due to no seed set because of increased ASI or there is no emergence of cob or reduced number of cobs per plant. Cobs/plot was noted to be one of the diverse parameters in teosinte derived maize lines. Tarun 83 had 4.7 cobs/plot whereas teosinte derived lines of Tarun 83 possessed minimum of 2.3 cobs/plot to maximum of 18.0 cobs/plot with overall mean of 9.3 cobs. The high range of cobs/plot is also reflected in the form of PCV and GCV which were recorded to be 45.2% and 42.9%, respectively. DMRHyd 1284-teosinte lines had range of variation from 5.7 to 14.7 cobs/plot with overall mean of 9.6. Cobs/plot in parental line DMRHyd 1284 was observed to be 5.7. The PCV and GCV for cobs/plot were 28.1and 24.0%, respectively. Pant12K 53 had least cobs/plot (1.3) whereas its teosinte derived lines had average cobs/plot of 6.8 with range from 1.3 to 15.7. PCV for cobs/plot was 53.7% whereas GCV was 48.8%. Such a diverse phenomenon for cobs/plot across the three teosinte derived lines indicates diversification through allelic reshuffling between maize and teosinte genomes. Grain yield/plot was observed to be the most diversified trait as indicated by high range of variation from minimum of 80.0g to maximum of 820.0g with high PCV and GCV of 58.9% and 58.7%, respectively in teosinte derived lines of Tarun 83. Overall yield/plot was 309.3 g whereas Tarun 83 had per plot yield of 120 g. The inbred line DMRHyd 1284 had plot yield of 69.7g whereas average grain yield of teosinte derived lines of DMRHyd 1284 was 371.4g with range of plot yield from 69.7 to 680.0 g. The PCV and GCV for yield/plot were 44.6% and 44.5%, respectively. Grain yield/plot in case of inbred line Pant12K 53 was 15.0 g whereas overall mean was 269.4 g with variation in yield from minimum of 15.0 to maximum of 670.0 g. Highest PCV and GCV of 72.5% and 72.5%, respectively were observed for yield/plot in Pant12K 53 derived lines.

The contribution of wild species to breeding cultivated species is well known. However, the characterization of adapted and wild populations is a fundamental aspect to increase the introduction of favorable genes in breeding programs (Laborda *et al*., 2005). While investigating hybrids between maize and perennial teosinte, Srinivasan and Brewbaker (1999) noted range of variations for many traits. Vigouroux *et al*., (2002) used SSR to identify genes of agronomic importance in maize under the influence of selection in the process of domestication. Lubberstedt *et al*., (1998) studied microsatellite variation in maize and suggested their use in detecting genetic variability in associated genotypes, which can be applied to maize and teosinte. In order to examine a broad range of genetic diversity for maize and shed light on the genetic basis of agronomic and domestication traits, Liu *et al*., (2016) developed near-isogenic introgression lines from ten teosinte accessions in the B73 background and determined genetic variation for flowering time and genetic architecture of traits specifically targeted by domestication. Further, they opined that introgression populations offer novel tools for QTL discovery and validation, as well as a platform for initiating fine mapping.

In the investigation, maize-teosinte crosses and backcrosses were made successfully. BC_1F_3 lines of three crosses exhibited significant variance for most of the characters. Mean, range, PCV and GCV for different characters analysed across the three populations indicated that teosinte derived lines were diversified probably because of the allelic reshuffling between maize and teosinte genome. Thus, teosinte can be used effectively for diversification as well enhancement of maize germplasm and domestication followed by selective breeding led narrow genetic base can be broaden using teosinte.

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