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Comparison of efficiency of F_1 and F_2 generations in identifying potential inbreds for improvement of a reference maize hybrid

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Abstract

The estimators namely, the relative frequency of favourable dominant alleles in a donor inbred (μG), the net improvement (NI) and the probability of net gain of favourable dominant alleles (PNG_g) were compared for their efficiency in ranking donors both within and between two methods, one using inbred and F_1 data and the other using inbred and F_2 data. Six donor maize inbreds (TCA21, TCA22, TCA24, CML32, IPA40 and SC7) were crossed to each of the parents of a reference maize hybrid, IPA2 × IPA34. Thirteen F_1's, 13 F_2's and 8 inbreds were evaluated in two locations in 1996 for grain yield. Inbreds with the highest and the lowest estimates of μG, NI and PNG_g were the same in both the methods. The efficiency of different estimators in identifying donors was highly consistent within a method and comparison of efficiency among them did not change with the method used. The ranking of donors as per μG and PNG_g varied greatly, while that as per NI remained the same with the method used. The donors with the closest relative relationship to either parent of the reference hybrid were the same in both the data sets. The differences in ranking of donors, different assessments of relative relationships of lines and different decisions on where to backcross prior to selfing may have stemmed from failure of assumptions such as no epistasis or difference in precision in the evaluation of different generations. The study indicated that direct selfing of the crosses IPA2 × TCA22 and IPA34 × SC7 would release an improved version of IPA2 and IPA34, respectively in a relatively shorter period.

Key words : *Zea mays*, donor, favourable alleles, relative relationship, backcross

Introduction

The identification of inbred lines with new favourable alleles for improvement of an elite single cross is an integral part of a pedigree maize (*Zea mays* L.) breeding programme Developments in quantitative genetic theory [1-5] provide methods for (a) identifying donor inbreds that contain favourable dominant alleles not already present in a reference hybrid, (b) determining the parent to which a particular donor inbred is relatively more closely related, and (c) obtaining the measure indicating whether to backcross prior to selfing. The above procedures have been applied in several earlier studies by using inbred and F_1 data [6-12]. In situations where the production of sufficient quantities of F_1 seeds is not possible in a crop, F_2 data could be used. Methods using inbred and F_2 data was first developed by Dudley [1] and the same was modified by Zanoni and Dudley [13]. Zanoni and Dudley [13] compared the efficiency of inbred and F_1 method (method I), and the inbred and F_2 method (method II) for relative frequency of new favourable alleles in donor inbreds, relative relationship (REL) of donor to either parent, and the measure (MB) indicating whether to backcross prior to selfing in an inbred improvement programme. No other reports on using the method II are currently available. Bernardo [4] proposed the net improvement statistic (NI) for both method I and method II. The estimator PNG_g, which is the probability of a net gain of favourable dominant alleles, was proposed by Metz [5] for identifying donor inbreds using method I. However, PNG_g could also be estimated by using method II after obtaining its component statistics μG, μD and μF based on inbred and F_2 data. No study using inbred and F_2 data for obtaining estimates of NI and PNG_g, and for comparing the ranking efficiency of NI and PNG_g between the method I and method II has been published. The objectives of the present study were : (i) to study the identification of potential donor inbred as per different estimators using inbred and F_2 data, and (ii) to compare the efficiency of the estimators in ranking the donor in inbreds and F_1, and inbred and F_2 methods

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Materials and methods

The parents consisted of eight second-cycle maize inbred lines. Two of these lines were the parents of an elite single cross, IPA2 × IPA34, which was considered as the 'reference hybrid' to be improved. The inbreds used as donors of new favourable alleles not already present in the reference hybrid were TCA21, TCA22, TCA24, CML32, IPA40 and SC7. All the eight inbred lines were developed at various stages of Indian maize breeding programme at Indian Agricultural Research Institute, New Delhi. The lines possessed substantial variation with respect to plant characters and yield related characters. The pedigree and origin of these inbreds are given in Table 1.

Table 1. Geographic origin, pedigree and seed characteristics of maize inbreds

Sl. No.	Source of population/cross	Country of origin	Races/germplasm involved	Seed characteristics of population	Inbred
1	A 64	Mexico, USA, Argentina, India and Caribbean region	Eto Amarillo, St. Coix4D, CBD Argentina, Antigua Basi and Coastal Tropical Flint	Yellow flint/semi-dent	IPA2
2	AD 609	Australia, Yugoslavia, India, Romania and Argentina	D1, D743, D751, A600, A603, Fitzroy, KS14C and KC2	Yellow flint/semi-flint	IPA40
3	MDR-1	Philippines, USA, Thailand, Guatemala and Caribbean region	Ph. DMR1, Ph.DMR5, Cupurico, Flint Compuesta	Yellow semi- flint	IPA34
4	MDR-1 × A64	As in 1 and 3	As in 1 and 3	yellow semi-flint	SC7
5	IPA1 × A64-FS-24	As in 1	As in 1	Yellow flint/semi-dent	TCA21, TCA22
6	IPA21 × A64-FS-24	As in 1 and 2	As in 1 and 2	Yellow flint	TCA24
7	POB28TS R(S2)	Unknown	Unknown	Yellow semi-flint	CML 32

Each of the donor inbreds was crossed on to IPA2 and IPA34. Each F_1 cross, including IPA2 × IPA34, was selfed once to produce F_2 seeds. The eight inbred lines (including IPA2 and IPA34), the 13 F_1 hybrids (including IPA2 × IPA34), and the 13 F_2's of these crosses were evaluated in separate but adjacent yield trials at two locations, Delhi and Karnal, during *kharif*, 1996. A randomized complete block design (RCBD) with three replications was used in each location for each trial. Both the inbred and F_1 trials consisted of two row plots while the F_2 trial was of four row plots. Each individual plot for each entry was 5m long with 0.75 m between rows and 0.25 m between plants. Planting was done in early July at both the locations. Recommended management and cultural practices were followed uniformly in all the trials.

Traits reported is grain yield (g plant^{-1}). Ten competitive random plants for inbreds and F_1's and forty for F_2's in each replication were tagged to record observations. Grain moisture at harvest was recorded and grain yield per plant was calculated in grams at 15% moisture after making adjustment for shelling percentage taken as 80. The average of the observations on the tagged plants for each entry was taken as the mean of an entry in a replication. Separate analysis of variance for grain yield were performed for each trial in each location. Combined analyses were performed after achieving homogeneity of error mean squares from the location-wise RCBD ANOVA.

The estimators used for identifying potential donor inbreds were μG, NI and PNG_g. The μG estimates the relative number of favourable dominant alleles present in a donor inbred but absent in a reference hybrid. This μG was computed using inbred and F_1 data following Dudley [3] and inbred and F_2 data following Zanoni and Dudley [13]. In each data set, μG can be calculated from four different equations. The choice of which equation to use is dependent upon the observed data. Different equations are necessary such that an allele frequency between zero and one can be estimated.

As proposed by Bernardo [4], the expression for NI is the maximum [(μG-μD), (μG-μF)]. When the component statistics μG, μD and μF were obtained using the inbred and F_1 data, the expression gave an estimate of NI for this data set but when the components were derived from inbred and F_2 data the expression was an NI for the latter data set. As per Metz [5], PNG_g is the maximum [μG/(μG + μD), μG/(μG + μF)]. The estimates of PNG_g were obtained for each data set after substituting the values of μG, μD and μF derived from the corresponding data set in this expression. Like μG, PNG_g also depends on gene frequency since its component statistics, μG, μD and μF are dependent upon gene frequency. In contrast, NI is independent of choice of equation regarding gene frequencies.

The REL of a donor inbred, I_w, to either of the parents I_1 and I_2 (IPA2 and IPA34, respectively in this study) was obtained by the expression, μC + μF-μD-μE, following Dudley [3] for method I and Zanoni and

Table 2. Mean grain yield of the lines and crosses (F_1's and F_2's) and inbreeding depression evaluated during 1996 at Delhi and Karnal (combined analysis)

Inbred line	*Per se*	F_1 × IPA2	F_2 × IPA2	Inbreeding* depression	F_1 × IPA34	F_2 × IPA34	Inbreeding depression**
TCA21	71.93	116.35	90.42	25.93	90.25	77.62	12.63
TCA22	84.02	124.53	105.83	18.7	109.82	90.47	19.35
TCA24	81.17	104.08	94.00	10.08	101.82	82.94	18.88
CML32	75.92	106.07	89.61	16.46	116.40	86.57	29.83
IPA40	81.00	98.56	92.31	6.25	89.83	81.61	8.22
SC7	49.04	120.55	94.64	25.91	91.63	75.22	13.41
IPA2	85.86				119.00	92.33	26.67
IPA34	55.60	119.00	92.33	26.67			
LSD (0.05)	6.12	6.25	5.91	6.08	6.25	5.91	6.08

*Inbreeding depression in F_2 for the donor × IPA2 crosses
**Inbreeding depression in F_2 for the donor × IPA34 crosses

Dudley [13] for method II. A significant positive value for REL indicates that I_w is closely related to I_1 and a significant negative value indicates that I_w is closely related to I_2. The MB was calculated as μG-μD, when I_w was relatively closer to I_1 and as μG-μF when I_w was relatively closer to I_2. This estimate was obtained by using values of μG and μD (μF) from inbred and F_1 method as per Dudley [3], and inbred and F_2 method following Zanoni and Dudley [13] to compare the decisions of whether to backcross prior to selfing. The estimators, REL and MB, are independent of gene frequencies at class j and k loci [3, 13]. Standard errors (SE) for all the estimators except PNG_g were calculated and the individual estimates were considered different from zero if they exceeded twice their standard error. Since PNG_g is a ratio of linear functions, its SE was not calculated due to the possibility of its improper approximation.

All the six donors were ranked based on the estimates of μG, NI and PNG_g in both the methods. Spearman's rank correlation coefficient (r_s) between the two data sets was calculated for each of these estimators. However, for both REL and MB, Pearson's simple correlation coefficient (r) was calculated to compare the method I and method II. Both types of correlation coefficients were tested for their significance as per Snedecor and Cochran [14].

Results and discussion

Mean grain yield from the combined analysis across the two locations were 106.84 g plant^{-1} for the F_1's 88.74 g plant^{-1} for the F_2's and 73.07 g plant^{-1} for the inbreds. The single cross hybrid IPA2 × TCA22, the F_2 hybrid (IPA2 × TCA22) F_2 and the inbred IPA2 were the highest yielding entries with grain yield 124.53, 105.83 and 85.86 g plant^{-1}, respectively (Table 2).

Estimates of relative frequency of favourable alleles (μG) were significantly different from zero for four inbred lines in method I, and for two lines in method II. The values for NI for grain yield were significant only for two inbreds using method I, while these were non-significant for all the inbreds using method II.

In both the methods, the inbreds TCA22, SC7 and IPA40 were unanimously ranked the best, the second best and the lowest (sixth), respectively by the estimators μG, NI and PNG_g (Table 3). The efficiency of μG and PNG_g in ranking the donors was exactly the same in each of the methods. However, only minor differences existed between NI and μG (or PNG_g) in ranking the donors TCA21 and CML32 in method I and TCA24 and CML32 in method II. Significant rank correlations were observed between μG and PNG_g (r_s = 1.00**), μG and NI (r_s = 0.94*) and NI and PNG_g (r_s = 0.94*) in both the methods (Table 4). This revealed that the efficiency of different estimators in identifying donors was much more similar within the data set and the comparative efficiency among them did not change with the data set used.

The estimator μG or PNG_g ranked TCA22, SC7, CML32 and IPA40 similarly, and TCA21 and TCA24 much more differently in the two data sets. This resulted in a non-significant rank correlation coefficient (r_s = 0.77) for μG (or PNG_g) between the two methods (Table 4). Similar results were obtained by Zanoni and Dudley [13] for μG between two data sets. In contrast, a significant rank correlation (r_s = 0.94*) was observed for NI between the methods in the present study.

Using inbred and F_1 data, CML32 and TCA24 had significant REL to IPA2, while SC7 had significant REL to IPA34 (Table 3). TCA22, the best donor, showed a negligible REL towards IPA2 while SC7, the second best donor, had significant REL to IPA34 based on this data set. Thus, the cross IPA34 × SC7 would enable the breeder extract an improved version of

Table 3. Different estimators of donor potential, relative relationship and measures indicating whether to backcross prior to selfing with respect of grain yield using the methods

Donor inbreds	μG	NI	PNG_g	μC + μF-μD- μE	μG-μD (μ F)
I. Inbred and F_1 method					
TCA21	6.96* (3)[d]	−1.33(4)	0.46(3)[d]	−10.97	−1.33
TCA22	11.05* (1)[d]	2.77(1)	0.57(1)[d]	0.42	−4.59
TCA24	0.83 (5)[d]	−7.46*(5)	0.09(5)[d]	12.87*	−8.59*
CML32	4.40* (4)[b]	−1.30(3)	0.44(4)[b]	25.46*	−1.30
IPA40	−1.94(6)[d]	−10.22*(6)	−0.18(6)[d]	6.40	−14.59*
SC7	9.06*(2)[d]	0.78(2)	0.52(2)[d]	−13.79*	0.78
II. Inbred and F_2 method					
TCA21	1.02(5)[d]	−5.99(5)	0.13(5)[d]	4.66	−11.13*
TCA22	13.41*(1)[d]	6.40(1)	0.66(1)[d]	−0.46	6.40
TCA24	2.29(3)[d]	−4.72(4)	0.25(3)[d]	8.14	−8.22
CML32	1.48(4)[b]	−3.28(3)	0.24(4)[b]	24.18*	−3.28
IPA40	0.65(6)[d]	−6.37(6)	0.08(6)[d]	8.86	−9.51
SC7	10.97*(2)[d]	3.95(2)	0.61(2)[d]	−8.58	3.95

$Significance not tested; Value within parenthesis indicates rank of the donor
μC + μF -μD-μE = Relative relationship; μG-μD (μF) = Measure indicating whether to backcross prior to selfing.
*Larger than 2 × S.E.; $b = \bar{q}_{j0}, \bar{q}_{k1}$ $d = \bar{q}_{k0}, \bar{q}_{k1}$

IPA34 via pedigree breeding much more easily as the cross IPA2 × TCA22 would do in extracting an improved version of IPA2.

Using the inbred and F_2 data, CML32 was the line with significant REL to IPA2, whereas, SC7 was the line with significant REL to IPA34. TCA22 had negligible REL towards IPA34 in this data set. Both the data sets showed that CML 32 and SC7 were the lines with the closest relationship to IPA 2 and IPA34, respectively. Thus, the cross IPA34 × SC7 would enable the breeder extract an improved version of IPA34 in a relatively shorter period as compared to IPA2 × TCA22. The REL of an I_w to the parent, I_1 or I_2 depends on differences in heterosis between $I_1 \times I_w$ and $I_2 \times I_w$ when inbred and F_1 data are used [3], and on differences in heterosis between $(I_1 \times I_w)$ F_2 and $(I_2 \times I_w)$ F_2 when inbred and F_2 data are used [13]. If, for every unit of decrease from the $I_1 \times I_w$ to $(I_1 \times I_w)$ F_2, there will be a proportionate decrease from $I_2 \times I_w$ to $(I_2 \times I_w)$ F_2 then a good amount of correlation (r) can be expected for REL between the two methods. As heterosis is expected to decrease by a coefficient of 0.5 after every generation of selfing, the fulfillment of this expectation will result in a significant positive correlation if there is little or no epistasis. In this study, the REL measures of the donors between the data sets showed a significant positive correlation ($r = 0.89^*$). However, in certain cases inbreeding depression from F_1 to F_2 was either greater or smaller relative to inbred yield when IPA2 was a parent than when IPA34 was a parent (Table 2). These resulted in a different assessment of the relative relationships for the two methods and suggested the presence of epistasis. Similar results were also reported by Zanoni and Dudley [13].

Using method I, the donors TCA24 and IPA40 had significant negative estimates of μG-μD showing the number of loci where IPA2 was unfavourable recessive and the donors were favourable dominants to be smaller than the number of loci where IPA2 was favourable dominant and the donors were unfavourable recessives (Table 3). All other donors had non-significant estimates of MB i.e. μG-μD (μF). In case of TCA24 and IPA40, a backcross to IPA2 prior to selfing of the crosses, IPA2 × TCA24 and IPA2 × IPA40, respectively, was indicated for a better possibility to extract an improved version of IPA2 from each of these crosses via pedigree breeding. However, the above two donors would not be chosen for improving the parents of the elite cross because they were not the best donors as per μG, NI and PNG_g. The two toppers, TCA22 and SC7, showed non-significant μG-μD and μG-μF estimates, respectively. Thus, the necessity of backcrossing prior to selfing was not indicated and the crosses IPA2 × TCA22 and IPA34 × SC7 could be selfed directly in order to isolate an improved version of IPA2 and IPA34, respectively via pedigree breeding (Fig. 1).

Table 4. Rank correlation coefficient (rs) of estimators within and between data sets (inbred and F_1 and inbred and F_2 data sets)

Estimator	μG	NI	PNG_g
μG	0.77	0.94*	1.00**
NI	0.94*	0.94*	0.94*
PNG_g	1.00**	0.94*	0.77

*P < 0.05; **P < 0.01

Diagonal value indicates rank correlation coefficient of an estimator between the two data sets.

The values above the diagonal indicate rank correlation coefficients between estimators in the inbred and F_1 data set

The values below the diagonal indicate rank correlation coefficients between estimators in the inbred and F_2 data set

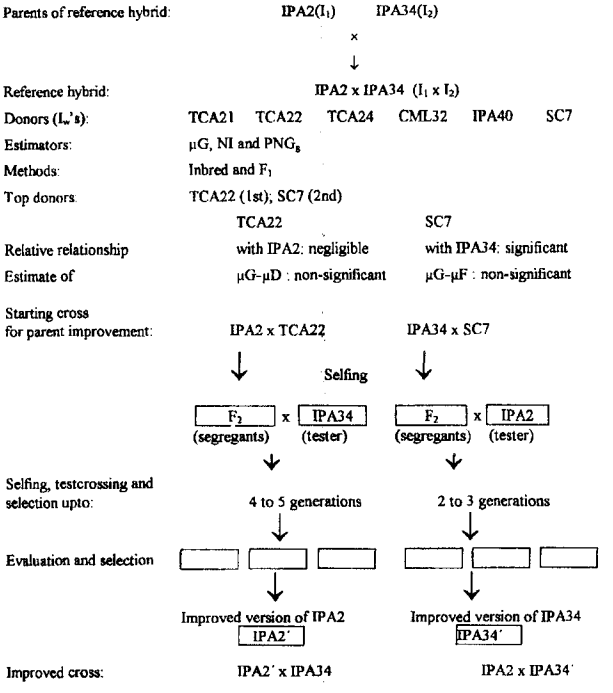

Fig. 1. Scheme for selection and utilization of donors with new favourable alleles to improve grain yield of a reference hybrid using different estimators in inbred and F_1 method

Using method II, the decisions to be made came out differently based on estimates of MB. The significant negative estimates of μG-μD for TCA21 suggested the need of atleast one backcross of IPA2 × TCA21 to IPA2 prior to its selfing. TCA22 and SC7 had non-significant μG-μF estimates and suggested direct selfing of the crosses IPA34 × TCA22 and IPA34 × SC 7, respectively, for extracting a line better than IPA34. However, an improved version of IPA34 could be isolated with much ease from IPA34 × SC7 as compared to that from IPA34 × TCA22 because of more REL of IPA34 to SC7 than to TCA22. The estimates of MB in method II were much more different from those in method I. These differences resulted in a non-significant correlation coefficient (r = 0.44). Low correlation of the values of MB between the two methods is obtained if the RELs of some donors (I_w) to I_1 or I_2 are assessed differently, and/or inbreeding depression of the parent × donor crosses and the elite cross is different. As evident from Table 3, there were differences in the RELs of some donors between the two methods. TCA21 was relatively more related to IPA34 in the method I while it was relatively related to IPA2 in method II. These differences in values of REL led to differences in the values of MB. It is evident from Table 2 that differences in inbreeding depression between the respective parent × donor cross and the elite cross existed for some of the donors which was as expected. As for example, the inbreeding depression from F_1 to F_2 was 12.63 for the cross IPA34 × TCA21, while it was 26.67 for the elite cross IPA2 × IPA34. The results of this investigation are in good agreement with Zanoni and Dudley [13].

The present investigation revealed that variation between the inbred and F_1, and inbred and F_2 methods existed in ranking a set of donor inbreds as per μG and PNG_g for grain yield. The reasons for this may be the failure of any of the assumptions, such as, no epistasis and the differential precision in assessing the inbreds, F_1's and F_2's. However, variation in the number of significant estimates of μG may be ascribed to differences in standard errors of μG between the two methods in addition to other reasons mentioned above. In general, all the three estimators μG, NI and PNG_g had similar efficiency in choosing the same topmost (i.e. TCA22 followed by SC7) and worst (IPA40) donors for yield in both the methods. Moreover, the efficiency of each of these three estimators was significantly correlated within a method and this similarity of their efficiency in ranking the donors didn't change with the data set used. However, there was no agreement between the two data sets in respect of donor ranks as per μG and PNG_g, and in the estimates of μG-μD (μF). The two methods were similarly efficient in ranking the donors as per NI and had more similarity in obtaining the relative relationship of the donors. Since μG is the only estimator for relative number of new favourable dominant alleles in a donor inbred and that rank correlation was non-significant between the two data sets for μG, evaluation of F_2's does not seem to be of any merit provided sufficient quantities of F_1 seed can be obtained. However, in a crop wherein enough F_1 seeds can not be produced, inbred and F_2 data would be informative in selecting the extreme lines (the best and the worst) with relative number of new favourable dominant alleles. Furthermore, evaluation in more number of environments may be helpful in obtaining smaller standard errors of the estimates.

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