Prospecting high oil in corn *(Zea mays L.)* germplasm for better quality breeding

Ambika Rajendran[1*], Dharampaul Chaudhary[2], Nirupma Singh[3]

[1,3]Scientist, ICAR-Indian Institute of Maize Research, Regional centre, New Delhi, India
[2]Senior Scientist, ICAR-Indian Institute of Maize Research, Regional Centre, Ludhiana, India
Present address: [1,3]Scientist, Division of Genetics, ICAR-Indian Agricultural Research Institute, Pusa Campus, New Delhi, India-110012;
** Corresponding author Email: rambikarajendran@gmail.com

Abstract

The present study was undertaken to assess genetic variability among oil content and agronomic traits in a set of corn inbreds. Oil content and plot yield ranged from 2.41 to 7.34% and 1.53 to 0.33kg, respectively. HKI-Tall-8-1-1, (TemperatexTropical(HO)QPM)-B-B-B-100-B-B, DMHOC4, (TemperatexTropical (HO)QPM)-B-B-B-57-B-B, HKI Talar, (TemperatexTropical (HO)QPM)-B-B-B-60-B-B and AF-04-b-5796-a-7-1-1 among 108 inbreds recorded above 6 per cent oil. AF-04-b-5796-a-7-1-1 was identified as an elite inbred with high oil and better yield for improvement of high oil corn. Wide range in performance was observed in oil and other phenotypic traits including yield. Principal Component analysis, regression and correlation coefficient facilitated sorting of inbreds and identification of related useful traits.

KeyWord *Zea mays. oil content, germplasm, quality*

Introduction

Corn is the main industrial crop worldwide also utilised for corn oil extraction. Corn oil is gaining its market among other traditional cooking oils, owing to its potential as healthy oil. Grain corn provides oil as by-product from left over kernel embryo/germ of starch wet mills. High oil corn is supposed to harbour more than 6 per cent oil in its kernel embryo/germ. Generally, high oil kernel in corn comes with reduced starch levels, smaller endosperm and reduced kernel size. Alexander and Lambert (1968) determined that the capacity of the plant to produce carbohydrates and synthesize oil is physiologically independent in the interval from 4 to 7% oil.

Genetic divergence guides the choice of parents for hybridization and identifies source of genes for a particular trait within the available germplasm for effective plant breeding. This is essential to meet the diversified goals such as breeding for quality and quantity. Significant variations in oil of nutritional importance exist among corn cultivars that can be exploited without any unfavourable effect on grain yield. Genetic analysis estimates the extent of variability among selected genotypes (Gasura, 2014). Since published work of high oil corn on genetic diversity is scanty, the present study was undertaken in germplasm of 108 inbreds to prospect high oil trait and to find out the nature and magnitude of genetic variability using principal component analysis and clustering.

Materials and Methods

Hundred and eight corn inbreds of different corn maturity types from the corn breeding program of Indian Institute of Maize Research were evaluated during the consecutive seasons (*Kharif* and *Rabi*) of 2011, 2012 and 2013 for agronomic performance and oil trait. Inbred lines were acquired from CIMMYT, India and National Bureau of Plant Genetic Resources, India. Various inbreds studied included twenty six exotic collections, twenty five indigenous lines and five CIMMYT CML lines. Experiment also comprised fifty two inbreds derived using pedigree method from different sources.

Agronomical trials

Plots of planting in Randomized Block Design with three replications consisted of six rows, 4 m long, spaced 75cm apart with 25cm spacing between plants within row. Intercultural operations like weeding, irrigation, mulching and earthing up were performed as and when necessary. The whole dose of recommended 80 kg N/ ha in the form of urea and 60 kg P_2O_5/ha as single super phosphate, 40 kg potassium/ha as murate of potash and 10 kg zinc as zinc sulphate was applied during planting as basal application. Fertilizer Urea was split two times; 1/3rd at knee high stage and the remaining 1/3rd at flowering. Pre-emergence application of weedicide Atrazine (0.75 kg/ha active ingredient) was used for weed control. Trials were kept weed-free by regular manual weeding. Other intercultural operations were done timely to raise the crop uniformly. The middle two rows were used for data recording in order to avoid contamination and ensure better selfing. Data was recorded on days to tasselling and silking, plant height (cm), ear placement (cm), ear length and diameter without husk (cm), number of grain rows, number of seeds per row, plot weight (kg), 100 grain weight (g) in ten randomly selected plants in each row of each replication.

Chemical analyses

To assess oil content, from each selfed cob, samples were obtained from the middle part of the cob to reduce variation that might result from sampling different parts of the same cob. The seeds (2g) were cleaned, shelled and ground prior to extraction. The ground samples were kept in a desiccator to avoid any contamination through moisture. All reagents were of analytical grade. The analyses were carried out in triplicates. Oil content was determined by the method of the AOAC (2009) using petroleum ether (40 - 60^{0}C) as solvent in a Soxhlet apparatus and again verified using an automatic solvent extraction system.

Statistical analysis

For the other field traits principal component analysis was run on the covariance matrix. The genotypes were grouped into clusters as per Ward's minimum variance method. Relationship between oil and other recorded traits were evaluated using linear regression and correlation. Statistical analysis was carried out using SAS and XLSTAT computer software.

Results and discussion

Analysis of traits for prospecting high oil among inbreds in acquired germplasm is represented in Table 1. Seven inbreds, HKI-Tall-8-1-1, Temperate x Tropical(HO) QPM-B-B-B-100-B-B, DMHOC4, Temperate x Tropical(HO)QPM)-B-B-B-57-B-B, HKI Talar, Temperate x Tropical(HO)QPM)-B-B-B-60-B-B and AF-04-b-5796-a-7-1-1, among 108 inbreds showed oil above 6 per cent to be identified as high oil corn lines (Table 2). Inbreds (TemperatexTropical (HO)QPM)-B-B-B-60-B-B (7.37%) and EC646076 (2.41%) had the highest and the lowest kernel oil content, respectively. Among high oil corn inbreds, line AF-04-b-5796-a-7-1-1 was found to be best in yield. CML-446-B-B and CML494 recorded highest plot weight. The range in plot yield and ear placement was four fold, while for oil content (%), plant length, hundred grain weight and number of seeds per row values varied by nearly three folds. Days to silking and tasseling, ear length and diameter without husk and number of grain rows varied by 30.5, 34.5, 10.1, 2.0 units and 8.0 respectively.

The relationship of the inbred lines between oil and other traits was significant for ear length, hundred grain weight and seeds per row. Regression analysis

Table 1. Simple analysis of traits in 108 corn inbred lines in study

Trait	Mean	Minimum	Maximum	Standard Error
Days to tasselling	58.29	40	70.5	0.56
Days to silking	62.71	46	80.5	0.61
Plant length (cm)	106.85	54	183.1	2.11
Ear placement (cm)	48.77	21.5	96.25	1.32
Ear length (cm)	12.09	7.4	18.1	0.18
Ear diameter (cm)	3.12	2.05	4.05	0.03
Number of grain rows	10.85	8	16	0.15
Plot weight (kg)	0.65	0.33	1.53	0.01
100 grain weight (g)	19.45	10.65	29.10	0.29
Number of seeds per row	19.69	11	31	0.43
Oil percent (%)	4.33	2.41	7.37	0.09

Table 2 -Mean performance of high oil inbreds for various traits recorded

Inbreds	DT	DS	PL	EP	EL	ED	NGR	PW	HGW	SPR	OP
DMHOC 4	56.5	60.5	124.7	83.625	10.4	3.1	10	0.58	20.45	13.5	6.11
Temperate x Tropical (H0)QPM-B-B-B-100BB	45.5	49.5	106	40.625	10.7	2.95	11	0.57	13.70	12	6.52
HKI-Tall-8-1-1	43	48	89.7	44.75	10.1	3.1	11	0.61	10.65	15	6.46
AF-04-b-5796-a-7-1-1	50.5	53	88.9	33.25	10.3	3.5	10	0.71	16.50	13	6.34
Temperate x Tropical											
(H0)QPM-B-B-B-60BB	48	51	85.5	32.8	11.25	3	12	0.70	11.00	14	6.44
HKI Talar	44	46	126.8	54	10.2	3.15	11	0.59	17.30	12.5	7.37
Temperate x Tropical (H0)QPM-B-B-B-57-B-B	48	51	171.3	86.375	10.45	3.05	13	0.57	20.80	22.5	5.99

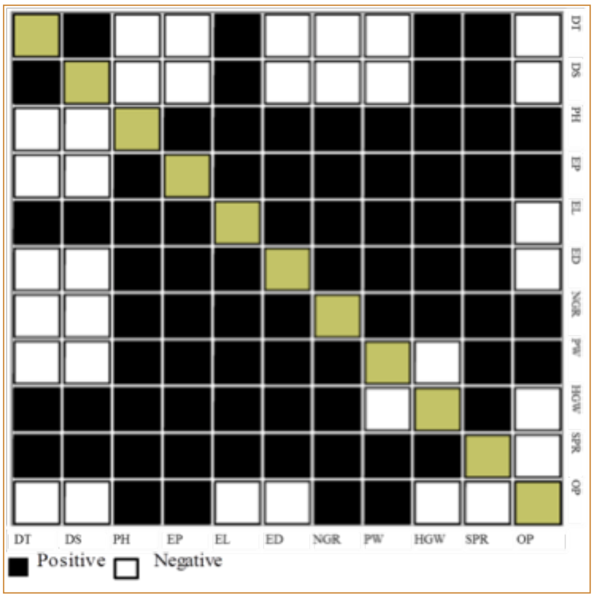
DT-Days to tasseling; DS-Days to silking; PL-Plant length (cm); EP-Ear placement (cm); EL-Ear length (cm); ED- Ear diameter (cm); NGR-Number of grain rows; PW-Plot weight (kg); HGW-100 grain weight (g); SPR-Number of seeds per row; OP-Oil percent (%)

indicated that with each 1 per cent increase in plot yield of grain, oil would increase by 0.01 per cent. Hundred grain weight, seeds per row, ear length and diameter, days to tasseling and silking were inversely related to oil percent with days to silking accounting for the highest 8% variation (Figure 1). The trend of ear length, hundred grain weight and seeds per row, positive contributors towards yield, was found to decrease as oil content increases. This might weaken the development of high yielding high oil corn. Nevertheless, in this case, plot yield had a non-significant association with kernel oil percent. Principal component analysis showed that 95.63% of the total variance (781.64) was contributed by the first three principal components of eleven characters under study simultaneously. The first, second and third principal components accounted for 70.91%, 15.77% and 8.95% of total variance (data not shown).

Agglomerative hierarchical clustering procedure with clusters determined by Ward's minimum-variance, grouped 108 inbred into three different clusters to identify diverse lines with respect to oil for use in high oil breeding. The first cluster comprised ten accessions which included one CIMMYT lines, three each indigenous lines, exotic lines and ear to row/pedigree derived lines. The second cluster comprised maximum 64 inbreds of sixteen indigenous, eleven exotic accessions, four CIMMYT lines and thirty three ear to row/pedigree derived inbred lines. Third cluster comprised twelve exotic lines, six indigenous accessions and sixteen ear to row/pedigree derived lines. Cluster 3 genotypes with highest mean value for ear length, ear diameter, number of grain rows, hundred grain weight, seeds per row and oil percent would be good resource for improvement directed towards high oil corn breeding to recombine these traits (data not shown).

Kernel oil in corn is a highly heritable trait (Mangolin *et al.* 2004). Corn commonly grown has a kernel oil concentration of about 4% on dry weight basis. Specialty high oil corn contains 6% or more oil on dry weight basis. Kernel oil content ranges between 1.2 and 20.0 on weight basis in corn (Singh et al. 2014). Development of corn cultivars with high oil without yield loss is a challenge in breeding programs (Mittelmann et al. 2011; Song et al. 2004). Multivariable factorial analysis identifies separately the independent factors which are most effective among recorded plant traits. Correlation coefficient and regression analysis among different traits aids indirect selection.

Although specialty traits like oil and starch are becoming more preferable in farmers, grain yield is still the most desired trait and quality traits that cannot be compromised for grain yield (De Groote et al. 2012).



DT-Days to tasseling; DS-Days to silking; PL-Plant length (cm); EP-Ear placement (cm); EL-Ear length (cm); ED- Ear diameter (cm); NGR-Number of grain rows; PW-Plot weight (kg); HGW-100 grain weight (g); SPR-Number of seeds per row; OP-Oil percent (%)

Figure 1. *Correlation map showing positive and negative relations of oil and other traits in corn inbred lines*

Low yield of high oil corn has directed to limited interest in high oil corn (Miller et al. 1981). The most important trait relation between plot yield and oil percent was not significant and the correlation value was positive yet weak (r = 0.08). Few cases depict positive associations between yield and oil content (Lambert et al. 1998; Alexander, 1999). However, frequent cases show negative association and non-relationship between oil content and yield (Ahuja and Malhi, 2008; Wassom et al. 2008) indicating that the yield has less to do with oil. Corn breeding program to increase oil content in the kernels should be designed to evade the grain yield decrease. Weak and favorable associations between traits indicate possibility of simultaneous selection for quality and yield traits. Mittelmann et al. (2011) suggested similar breeding strategy for quality breeding in corn. Ranking of lines for oil and yield also had no apparent relationship. Most of the top ten lines for yield were in the lower half of the rank for oil and *vice versa*. This suggests that it might be possible to increase both yield and oil concentration using improved inbreds from this germplasm similar to the conclusions of Wassom et al. (2008).

Traits with relatively greater weight in the first principal component had highest contribution to the total variability and responsible for cluster formation (Chozin, 2007; Mujaju and Chakuya, 2008; Ali et al. 2011). In our research, the first principal component was mainly due to variables plant length, ear placement, days to silking and tasseling. Selection of genotypes for transgressive breeding may be based on traits which contribute maximum to variation in principal component. The positive and negative loading shows the presence of positive and negative correlation between the components and variables (Hailegiorgis et al. 2011). There exists relationship of increased oil with reduced plant height and ear height, early flowering, lighter kernel weight, reduced ear length and ear width (Singh et al. 2014). Therefore, the variability in the present material due to these traits is also very much important for selection of appropriate parents. The existence of genetic variability and the prospect of selection for oil content in corn have been recognised in number of studies (Song et al. 1999). Regarding traits of our interest, principal component analysis of oil and yield confirmed that overall amount of variation in this set of inbreds under study was not enough/scanty to be utilized for breeding to improve oil content as well as grain yield. It is a fact that maximum the variation maximum the heterosis. The result of amount of variation accounted by the covariates (oil per cent and yield) in principal component analysis would have been better if the material chosen for study had been rich/appropriate in terms of yield or trait of our interest oil. The choice of material for prospecting was random apart from any set criteria. AF-04-b-5796-a-7-1-1 is the sole elite inbred identified with high oil and better yield suggested for improvement of high oil corn.

Hybridization between genotypes of different clusters with high cluster mean will result into transgressive segregants with high yield potential (Sisodia et al. 1983). Clustering of genotypes into groups is mainly attributed by cumulative effects of individual traits. Generally crosses involving parents belonging to most distant clusters are expected to produce maximum heterosis. Breeder can also perform hybridization among the genotypes of the same cluster having high intra-cluster distance to get heterosis and variation. Again for improving several desirable traits, combination breeding can be done among the genotypes of different clusters. Utilisation of high oil lines as pollinators to harness xenia effect is a well-studied aspect in high oil corn breeding. Xenia effect increases oil in germ as well as size of germ without affecting yield (Thomison et al. 2002; Liu et al. 2010). Such pollinators are crossed with elite hybrids as female in a breeding strategy to develop superior high oil top cross cultivars.

There is commercial interest in increasing oil concentration in maize grain; the development of cultivars that are superior for both yield and oil concentration is expected to be feasible as there was no association between nutritional quality and yield.

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