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Characterization and genetic studies for grain iron content and agronomic traits in Recombinant Inbred Lines (RILs) population of maize (*Zea mays* L.)

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Abstract

Maize, a vital cereal crop, serves as a staple food, animal feed, and industrial ingredient. An experiment was conducted during *Rabi* 2018 and Spring 2019, to assess genetic variability in RIL population of the cross GLY 6 (low iron content, strong agronomic traits) and CML 296 (high iron content). Variability was observed in kernel iron content and 11 agronomic traits, indicating differences among RILs. Moderate genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) for kernel iron content were observed across seasons. High heritability and moderate genetic advance as percentage of mean (GAM) were recorded. Traits related to yield had narrow GCV and PCV, suggesting potential for improvement through selection. Moderate to high heritability and GAM were noted for many traits, indicating less environmental influence and potential scope for direct selection to enhance micronutrient content and yield. Yield per plot showed negative correlation with kernel iron content in both seasons. Pooled analysis showed no significant seasonal impact on grain iron concentration among 120 RILs. Transgressive segregants indicates scope for nutrient enrichment and higher grain yielding potential.

Keywords: Maize, genetic variability, heritability, GAM and Transgressive segregants

INTRODUCTION

Micronutrient deficiency in daily dietary intake, leading to hidden hunger, is one of the major health concerns worldwide, especially in resource poor developing countries of Africa and South and South East Asia (Bouis *et al.*, 2019). Iron (Fe) and Zinc (Zn) are essential micronutrients required for normal growth and development of animals (Neeraja *et al.*, 2017). Deficiency of Fe and Zn leads to retarded growth, weak immunity, sterility, reduced cognitive response, morbidity and even death in acute cases (Haas and Brownlie, 2001). Dietary diversification, food fortification and supplementation often used to address the problem of malnutrition, but these strategies have shown limited success in resources poor developing countries (Pfeiffer and McClafferty, 2007). Development of micronutrient rich crop varieties through breeding approaches, a process known as biofortification, provides sustainable and cost effective solution to overcome hidden hunger (Mehta *et al.*, 2020). Maize is one of the three topmost cereals being cultivated and consumed across the globe (Shiferaw *et al.*, 2011). It is the staple food crop for more than 4.5 billion people worldwide, and constituted 38% of the food calories in Africa, 30% in America and 6.5% in Asia, thus, justifying its significance in food security (Prasanna *et al.*, 2020). Apart from calories, maize is also an important source of fatty acids, dietary fibers, carotenoids, anthocyanins and phenolics, providing health benefits (Zhang *et al.*, 2021). However, baseline content of Fe in maize is 30µg/g, which is not sufficient to meet the estimated average requirement (EAR) of 1460 µg/day for Fe

(Ibrahim *et al.*, 2021). The target levels in maize grain were set at 52 μ g/g for Fe, therefore, improving Fe content in maize assumes greater significance (Andersson *et al.*, 2017).

Breeding for high yielding and micronutrient rich crop varieties depends greatly upon the exploitable level of genetic variability in available germplasm and the amount of heritable variation (Jaiswal et al., 2019). Genotypic and phenotypic coefficient of variation (GCV and PCV) helps to determine the amount of variability in a population for target traits. Estimates of heritability and genetic advance determine the efficiency by which the genotypic variability can be utilized through selection (Rai et al., 2021). The knowledge of interrelationship between traits is useful for developing selection criteria for their simultaneous improvement (Phuke et al., 2017). Besides, proper characterization and understanding environment is considered vital for selecting genotypes with high grain yield and micronutrient content (Ekpa et al., 2019). Thus, for selection of genotypes from a recombinant inbred lines (RILs) population, assessment of variability, heritability and expected genetic advance is necessary. In the present study, we investigated genetic variability, heritability, genetic advance, correlation and genotype × environment interaction (G × E) for grain Fe content and agronomic traits in RILs population of maize.

MATERIALS AND METHODS

Experimental material and field trials: A population of 120 F_6 RILs was developed by single seed descent (SSD) method from the cross GLY6 (low Fe) × CML296 (high Fe) at Main Maize Breeding Research Station, Godhra, India. The RILs and their parents were grown in Randomized Complete Block Design with two replications during Rabi, 2018 and Spring, 2019 at Research Farm, College of Agriculture, Vaso, India (22°67' N and 72° 77' E, 30.6 above mean sea level). Each genotype was planted in single row of 4 m length with 60 cm inter-row and 15 cm intra-row spacing. The recommended agronomic practices relevant to maize crop were followed throughout the crop period.

Data recording for Agronomic traits and Biochemical analysis: Five plants per RIL in each replication were randomly selected to record the data on ten agronomic traits. For estimation of kernel Fe, dried seeds of five cobs in each replication were bulked and two representative samples were drawn as technical replicates. The grains were powdered and Fe was extracted by double digestion method and concentrations of Fe were estimated using Atomic Absorption Spectroscopy (AAS). All the biochemical analysis were conducted at Centre of Excellence of Biotechnology Laboratory, Anand, India.

Statistical analysis: The recorded data of all the traits were analyzed to calculate GCV, PCV, heritability (broad sense), genetic advance (GA), genetic advance as

percent of mean (GAM), correlation and stability. Analysis of variance (ANOVA) with two season data for all the traits was performed using Statistical Analysis System (SAS) version 9.1. Pooled analysis of variance for iron content across two seasons was carried out by following Eberhart and Russell (1969) stability model. PCV and GCV were estimated using the formula given by Burton (1952), Heritability (broad sense) was calculated as the ratio of genotypic variance to the total variance (Lush, 1940). The computation and categorization of GA and GAM was carried out according to the method given by Johnson *et al.* (1955). Correlation between grain Fe and agronomic traits was determined as per the method suggested by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Analysis of variance: The mean sum of square due to various source of variation for grain iron content and agronomic traits for RILs obtained from GLY6×CML296 for both seasons were presented in Table 1. The results revealed the presence of adequate amount of genetic variability for all the characters studied in all genotypes indicating presence of significant differences among the RILs. The RILs are the product of continuous hybridization and repeated selfing of individual F₂ seeds by following single seed descent method. Entire genetic variation generated due to crossing over is captured in the RILs, which serves as a good source of variability. Some of the RILs also displayed transgressive segregation for all the studied traits. Gu et al. (2015), Gokulakrishnan et al. (2021) also reported similar results of high genotypic variation for grain iron concentration in RIL population at China. The results were in conformity with results of Simic et al. (2009) at Croatia where high genetic variation for kernel iron content was reported in F_{4} population of maize. High genetic variation for yield traits like ear diameter, ear length, kernel row number, cob diameter, ear weight, grain weight and 100 kernel weight were reported in F_{2.3} population of maize by Zhao and Su, (2019).

Mean performance RILs: The range of variation and mean iron content and 11 other agronomic traits studied for both seasons are presented in Table 2. For Rabi, 2018, the range of variation for kernel iron content was 17.75 mg/ kg (RIL-73) to 37.93 mg/kg (RIL-118) with an average of 26.28 mg/kg. For Spring, 2019, the range of variation for grain iron content was 19.29 mg/kg (RIL-26) to 38.19 mg/ kg (RIL-94) with an average of 26.61mg/kg. The range of variation was found to be highest for plant height for both the seasons viz. 82-174 cm with an average of 118.91 cm for Rabi, 2018 and 81-171 cm with an average of 124.7 for Spring, 2019. The range and average values reflect the amount of variability existing in the population. Hence, the study revealed the existence of significant amount of genetic variability for all the studied traits in RILs. This extensive range in performance for different traits among RILs was due to their unevenness in adjustment to different agro-climatic conditions. By comparing mean

| Source | Df | Days te tasse | | Days t silk | | Plant I (cr | | | r of cob plant | | veight g) | | length :m) |
|------------|-----|---------------------|----------------|---------------------|----------------|---------------------|----------------|---------------------|-------------------|---------------------|----------------|---------------------|----------------|
| | | <i>Rabi</i> 2018 | Spring 2019 | <i>Rabi</i> 2018 | Spring 2019 | <i>Rabi</i> 2018 | Spring 2019 | <i>Rabi</i> 2018 | Spring 2019 | <i>Rabi</i> 2018 | Spring 2019 | <i>Rabi</i> 2018 | Spring 2019 |
| Replicates | 1 | 0.0042 | 0.94 | 0.017 | 0.10 | 129.07 | 54.15* | 0.004 | 0.15 | 17.33* | 138.47* | 0.165 | 0.04 |
| Genotypes | 119 | 7.53* | 10.89* | 11.53* | 9.93* | 680.97* | 685.45* | 0.217* | 0.14* | 79.68* | 85.01* | 3.53* | 3.03* |
| Error | 119 | 1.92 | 2.5 | 3.1 | 2.1 | 15.8 | 15.7 | 0.079 | 0.02 | 3.54 | 8.5 | 0.65 | 0.4 |
| R^2 | | 0.97 | 0.97 | 0.96 | 0.97 | 0.99 | 0.99 | 0.73 | 0.68 | 0.96 | 0.96 | 0.98 | 0.98 |
| CV(%) | | 2.56 | 2.9 | 3.06 | 2.53 | 3.34 | 3.18 | 2.8 | 2.66 | 1.81 | 2.84 | 5.94 | 4.79 |
| CD(0.05) | | 2.74 | 3.13 | 3.48 | 2.87 | 7.8 | 7.85 | 0.56 | 0.28 | 3.73 | 5.77 | 1.59 | 1.25 |
| S.E Mean | | 0.98 | 1.12 | 1.24 | 1.02 | 2.81 | 2.80 | 0.194 | 0.1 | 1.33 | 2.06 | 0.57 | 0.45 |

Table 1. Mean squares for yield, iron concentrations and other agronomic traits measured in RIL population ofcrossGLY6 ×CML296 during Rabi, 2018 and Spring, 2019.

| Source | Df | | width cm) | Number row p | of kernel er cob | | ber of per row | 1000 k wei (g | ght | | oer plot (g) | | on g/kg) |
|------------|-----|---------------------|----------------|---------------------|---------------------|---------------------|-------------------|---------------------|----------------|---------------------|-----------------|---------------------|-----------------------|
| | | <i>Rabi</i> 2018 | Spring 2019 | <i>Rabi</i> 2018 | Spring 2019 | <i>Rabi</i> 2018 | Spring 2019 | <i>Rabi</i> 2018 | Spring 2019 | <i>Rabi</i> 2018 | Spring 2019 | <i>Rabi</i> 2018 | S <i>prig</i> 2019 |
| Replicates | 1 | 0.15* | 0.002 | 0.15 | 0.017 | 1.15 | 1.24* | 160.07 | 161.7* | 0.04* | 0.05* | 1.08* | 2.42* |
| Genotypes | 119 | 3.28* | 3.21* | 0.38* | 0.45* | 5.48 | 5.33* | 366.7* | 399.4* | 0.05* | 0.06* | 21.84* | 21.2* |
| Error | 119 | 1.2 | 1.1 | 0.12 | 0.12 | 0.22 | 0.92 | 14.2 | 21.1 | 0.008 | 0.009 | 3.5 | 3.4 |
| R^2 | | 0.99 | 0.98 | 0.81 | 0.89 | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 | 0.99 | 0.97 |
| CV(%) | | 9.6 | 9.03 | 2.97 | 3.01 | 2.01 | 4.06 | 1.75 | 0.67 | 4.95 | 5.10 | 7.11 | 7.2 |
| CD(0.05) | | 2.17 | 2.08 | 0.68 | 0.69 | 0.92 | 1.89 | 7.46 | 2.88 | 0.177 | 0.19 | 3.7 | 3.7 |
| S.E Mean | | 0.77 | 0.74 | 0.24 | 0.25 | 0.33 | 0.68 | 2.66 | 1.03 | 0.063 | 0.07 | 1.33 | 1.3 |

* Significant at 0.05 probability level,** and 0.01 probability level

performance of different traits, it was found that there was a significant difference or variation for all quality traits. Similar results of high variability as explained by large values of range for maize kernel iron content was reported by Gu *et al.* (2015). High variability for yield traits such as ear diameter, ear length, kernel row number, cob diameter, grain weight ear weight, and 100 kernel weights in maize were also reported by Zhao and Su, (2019).

Genotypic and phenotypic coefficient of variation: Variability analysis for iron content and other agronomic traits of mapping population of cross GLY6 ×CML296 for Rabi, 2018 and Spring, 2019 were furnished in Table 3. For Rabi'2018 and Spring '2019, the genetic coefficient of variation (GCV) was low (9.41% and 9.51% respectively) while phenotypic coefficient of variation (PCV) was medium (11.79% and 11.93% respectively) for kernel iron content. Low GCV was reported for days to 50% tasseling and silking, cob weight, cob length, number of kernel rows per cob, number of kernels per row, thousand kernel weight and single plot yield for both seasons while plant height, number of cob per plant had expressed medium GCV for both the seasons. Cob width had exhibited medium GCV during Rabi' 2018 and low GCV during Spring, 2019. PCV was low for days to 50% tasseling and

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silking, cob weight, cob length, number of kernels per row, number of kernel rows per cob, thousand kernel weight and plot yield while medium PCV was recorded for plant height and cob width for both the seasons. Number of cob per plant was found to display high PCV during both the seasons.

In the present investigation, for kernel iron content, low - GCV coupled with moderate PCV was observed during both the seasons. These coefficients of variation indicate the existence of considerable amount of variation for these characters. These findings were in conformity with the results reported by Jaiswal et al. (2019) where medium GCV and PCV were found for iron. High GCV and PCV for kernel iron content in sweet corn was reported by Suhaisini et al. (2016). The co-efficient of variation calculated at phenotypic and genotypic levels are being used to evaluate the variability among different traits. Moderate GCV and PCV reported in present study for plant height and narrow GCV and PCV reported for days to flowering, cob length, cob weight, cob width, number of kernels per row, number of kernel rows per cob, thousand kernel weight and single plot yield was in accordance with results of Mani and Deshpande (2016). Moderate values of 100 grain weight, ear girth and number of grains per

Table 2. Descriptive Statistics for agronomic traits and iron concentrations in RIL population of cross GLY6 ×CML296 sown in *Rabi*, 2018 and *Spring*, 2019

| Traits | MEAN | (±S.E) | RAN | NGE | | IDARD ATION | | IPLE ANCE | SKEV | VNESS | KUR | TOSIS |
|------------------------------|-----------------------|-----------------|-----------------------|-----------------------|-----------------------|-----------------|-----------------------|-----------------|-----------------------|-----------------|-----------------------|-----------------|
| _ | <i>Rabi</i> , 2018 | Spring, 2019 | <i>Rabi</i> , 2018 | Spring, 2019 | <i>Rabi</i> , 2018 | Spring, 2019 | <i>Rabi</i> , 2018 | Spring, 2019 | <i>Rabi</i> , 2018 | Spring, 2019 | <i>Rabi</i> , 2018 | Spring, 2019 |
| Days to 50% tasseling | 54.18± 0.18 | 54.29± 0.21 | 48-61 | 48-61 | 1.94 | 2.33 | 3.76 | 5.44 | 0.15 | 0.86 | 1.59 | 1.99 |
| Days to 50% silking | 57.61± 0.22 | 57.35± 0.20 | 52-69 | 52-63 | 2.4 | 2.23 | 5.77 | 4.96 | 0.29 | -0.28 | 3.11 | -0.31 |
| Plant height (cm) | 118.91± 1.68 | 124.68± 1.69 | 82- 174 | 81- 171 | 18.45 | 18.51 | 340.49 | 342.73 | 0.45 | -0.03 | 0.38 | 0.19 |
| No. of cob per plant | 1.154± 0.03 | 1.12± 0.024 | 1-2.5 | 1-2 | 0.33 | 0.27 | 0.11 | 0.07 | 2.09 | 2.38 | 3.43 | 4.10 |
| Cob weight(g) | 103.93± 0.58 | 102.71± 0.59 | 91- 115.5 | 91- 113 | 6.31 | 6.52 | 39.84 | 42.50 | -0.47 | 0.01 | -1.01 | -1.32 |
| Cob length(cm) | 13.56± 0.212 | 13.19± 0.11 | 10.9- 16.1 | 10.9- 16 | 1.33 | 1.23 | 1.76 | 1.52 | 0.19 | 0.76 | -1.16 | -0.09 |
| Cob width(cm) | 11.39± 0.12 | 11.61± 0.12 | 9.45- 13.65 | 9.45- 13.55 | 1.28 | 1.27 | 1.64 | 1.60 | -0.068 | -0.21 | -1.26 | -1.19 |
| No. of kernel row per cob | 11.66± 0.04 | 11.54± 0.04 | 11-12 | 11-12 | 0.44 | 0.47 | 0.19 | 0.23 | -0.67 | -0.17 | -1.35 | -1.89 |
| No. of kernel per row | 23.23± 0.15 | 23.62± 0.15 | 20.35- 27.1 | 20.35- 26.9 | 1.66 | 1.63 | 2.74 | 2.66 | 1.014 | -0.11 | -0.79 | -0.5 |
| 1000 kernel weight(g) | 215.6± 1.24 | 217.75± 1.29 | 193.5- 243.5 | 194- 242 | 13.54 | 14.13 | 183.33 | 199.69 | 0.27 | 0.01 | -1.077 | -1.26 |
| Yield per plot(kg) | 1.81± 0.02 | 1.86± 0.02 | 1.59- 2.24 | 1.575- 2.25 | 0.16 | 0.167 | 0.03 | 0.028 | 1.16 | 0.63 | 0.88 | -0.03 |
| lron (mg/kg) | 26.28± 0.30 | 26.61± 0.29 | 17.75- 37.93 | 19.2 - 38.1 | 3.30 | 3.26 | 10.92 | 10.61 | 0.35 | 1.13 | 0.82 | 1.39 |

Table 3. Variability analysis for agronomic traits and iron concentrations of RIL population of cross GLY6 ×CML296 sown in *Rabi*, 2018 and *Spring*, 2019

| Traits | GCV | /(%) | PC | V(%) | h²B | (%) | G | iA | G | AM |
|---------------------------|-----------------------|-----------------|-----------------------|-----------------|-----------------------|-----------------|-----------------------|-----------------|-----------------------|-----------------|
| | <i>Rabi</i> , 2018 | Spring, 2019 |
| Days to 50% tasseling | 2.5 | 3.08 | 3.59 | 4.24 | 49.3 | 52.81 | 1.98 | 2.5 | 3.65 | 4.61 |
| Days to 50% silking | 2.91 | 2.82 | 4.22 | 3.78 | 47.54 | 55.4 | 2.38 | 2.48 | 4.13 | 4.32 |
| Plant height (cm) | 12.52 | 11.98 | 12.96 | 12.39 | 93.34 | 93.43 | 29.64 | 29.75 | 24.92 | 23.86 |
| No. of cob per plant | 18.65 | 17.93 | 30.74 | 21.93 | 36.8 | 66.85 | 0.27 | 0.34 | 23.30 | 30.2 |
| Cob weight(g) | 4.84 | 4.92 | 5.17 | 5.68 | 87.75 | 75.0 | 9.72 | 9.01 | 9.35 | 8.77 |
| Cob length(cm) | 7.23 | 7.09 | 9.35 | 8.57 | 59.63 | 68.87 | 1.56 | 1.59 | 11.49 | 12.12 |
| Cob width(cm) | 7.31 | 7.22 | 12.08 | 11.57 | 36.62 | 39.0 | 1.04 | 1.08 | 9.11 | 9.29 |
| No. of kernel row per cob | 2.52 | 2.87 | 3.89 | 4.16 | 41.93 | 47.82 | 0.39 | 0.47 | 3.63 | 4.09 |
| No. of kernel per row | 5.69 | 5.13 | 6.03 | 6.55 | 88.85 | 61.51 | 2.57 | 1.96 | 11.04 | 8.29 |
| 1000 kernel weight(g) | 5.03 | 5.16 | 5.32 | 5.57 | 89.21 | 85.67 | 21.09 | 21.41 | 9.78 | 9.83 |
| Yield per plot(kg) | 6.87 | 6.66 | 8.47 | 8.39 | 65.71 | 63.01 | 0.21 | 0.20 | 11.46 | 10.89 |
| Iron (mg/kg) | 9.41 | 9.51 | 11.79 | 11.93 | 63.59 | 63.58 | 4.06 | 4.0 | 15.46 | 15.63 |

PCV- phenotypic coefficient of variation, GCV-genotypic coefficient of variation, GAM-genetic advance as per cent mean, GA-genetic advance, h²-heritability

cob were also reported by Singh *et al.* (2019). Moderate GCV and PCV indicate reasonable variability among the genotypes with respect to these characters and therefore there is a scope for improvement of these traits through selection in future generations. If the variation between GCV and PCV values is very less, it indicates imperfect role of environment Similar results were reported by Bello *et al.* (2012).

It was also found that GCV was lower than PCV for most of the characters studied. Falconer (1960) also studied the relation between genotypic to phenotypic variations, according to which, the higher the genotypic to phenotypic variation ratio, the more the heritability of the trait and if the ratio is small there is indication of more control of the environment on the phenotypic expression of the character.

Heritability and genetic advance: Heritability for kernel iron content was scored high for both the seasons (63.59% and 63.58% respectively), while the genetic advance as percent mean (GAM) was found to be moderate (15.46% and 15.63% respectively). Heritability of plant height, cob weight, number of kernels per row, thousand kernel weight and single plot yield was high for both the seasons. Medium heritability was reported for days to 50% tasseling, days to 50% silking, cob width and number of kernel row per cob for both the seasons. The GAM was high for plant height and number of cobs per plant for both the seasons. GAM was reported to be moderate for cob length for both the seasons. Heritability value is an important indication of progress of selection. The GCV along with heritability estimate provides dependable estimate of the amount of genetic advance expected through phenotypic selection (Wright, 1921). Simic et al. (2012) reported medium heritability for kernel iron concentration in maize. For 100 grain weight, cob length and grain yield per plant, high heritability and high genetic advance had been reported (Hemavathy et al., 2008). According to Bello et al., (2012), the information of the amount of genetic variability, heritability and genetic gain helps the plant breeder in fixing criteria for using it in breeding programmes. High heritability and genetic advance for different characters were reported by earlier researchers (Rafig et al., 2010) in maize. High heritability together with high genetic advance as percent mean indicates that the traits are predominantly under the control of additive and /or additive × additive gene interactions and direct selection would be preferred for character improvement.

Test of normality: In order to know the frequency distribution of a segregating generation and their genetic interactions for a particular trait, skewness and kurtosis was estimated (**Table 2**). The skewness obtained from the frequency distribution of the present study revealed that in both the seasons, kernel iron content showed positive skewness. Cob width and number of kernel

row per cob showed negative skewness during both the seasons while positive skewness was observed for days to 50% tasseling, number of cob per plant, cob length, 1000 kernel weight and yield per plot during both the seasons. Skewness explains how much the departure of a distribution from symmetry is. If the skewness is positive it indicates the complementary epistatic gene action for the trait studied and the genetic gain is slower with mild selection and faster with intensive selection. Skewness when negative indicates that duplicate epistasis gene action and the genetic gain is faster with mild selection and rapid with intense selection (Snape and Riggs, 1975). Frequency distribution study in both the seasons revealed positive kurtosis for kernel iron content.. Cob weight, cob width, number of kernel row per cob and number of kernel per row displayed negative kurtosis while positive kurtosis was observed for days to 50% tasseling, plant height and number of cobs per plant for both the seasons. The traits exhibiting leptokurtic distribution were assumed to be under the control of few segregating genes while traits expressing platykurtic distribution were under the control of many genes with complementary gene action. If the value is zero (mesokurtic), it indicates normal distribution. For the characters showing platykurtic distribution, the genetic gain would be faster if selection was made intensively.

Correlation between grain Fe content and agronomic traits: During both the seasons days to 50% tasseling and days to 50% silking associated significantly positively with kernel iron concentration (0.19**). Yield per plot is significantly negatively correlated with kernel iron (-0.28* and -0.28* respectively) content during both the seasons. Number of cob per plant, number of kernel rows per cob have exhibited positive association though non-significantly correlated with kernel iron content. However, plant height, cob length, cob weight, number of kernels per row, cob width and 1000 kernel weight were found to show negative nonsignificant correlation with kernel iron content (**Table 4**).

Correlation is defined as the linear association of breeding values of individuals for two traits. Strong and positive correlation between different traits allow breeders to advance the correlated traits concurrently. Due to the enhanced concentration of total seed weight resulting from increased starch accumulation, the mineral concentration in the seed decreases. Consequently, there exists research potential for enhancing kernel mineral concentration without compromising grain yield. Chakraborti *et al.* (2009) reported negative significant correlation of grain iron content with flowering as well as yield.

Pooled analysis of variance: Season wise ANOVA (**Table 5**) revealed non-significant variation for grain iron concentration in both the seasons among 120 RILs suggesting nonsignificant role of environment in

| Table 4. Correlation between kernel iron and certain agronomic traits of RIL population of cross GLY6 ×CML296 |
|---|
| during Rabi' 2018 and Spring' 2019 |

| Traits | Correlation between kernel iron and certain agronomic traits of RIL popula cross GLY6 ×CML296 | | | |
|---------------------------|--|--------------|--|--|
| | Rabi, 2018. | Spring, 2019 | | |
| | lron (mg/kg) | lron (mg/kg) | | |
| Days to 50% tasseling | 0.197(0.032)** | 0.02(0.82) | | |
| Days to 50% silking | 0.189(0.039)** | -0.02(0.81) | | |
| Plant height (cm) | -0.172(0.061) | -0.003(0.98) | | |
| No. of cob per plant | 0.077(0.41) | 0.02(0.86) | | |
| Cob weight(g) | -0.059(0.53) | 0.08(0.38) | | |
| Cob length(cm) | -0.105(0.25) | 0.11(0.24) | | |
| Cob width(cm) | -0.019(0.831) | 0.08(0.38) | | |
| No. of kernel row per cob | 0.116(0.21) | -0.08(0.41) | | |
| No. of kernel per row | -0.097(0.29) | -0.02(0.82) | | |
| 1000 kernel weight(g) | -0.071(0.45) | 0.07(0.48) | | |
| Yield per plot(kg) | -0.28(0.002)* | -0.08(0.38) | | |
| Iron (mg/kg) | 1(-) | 1 | | |

Correlation coefficients with p value in bracket;

* Significant @ 1%, ** Significant @ 5%, *** Significant @10%

| Table 5. Combined analysis of variance for grain Iron concentration of 120 RILs grown during Rabi' 2018 and |
|---|
| Spring' 2019 |

| Source | df | lron (mg/kg) |
|------------------|-----|--------------|
| Replicates/Year | 2 | |
| Season | 1 | 52.46 |
| Genotypes | 119 | 25.20 |
| Season×Genotypes | 119 | 18.01 |
| Pooled Error | 238 | 0.251 |
| CV% | | 1.93 |

performance of genotypes. Non-significant interaction suggests that genotypes react differently to variable environmental conditions and it is very difficult to predict the performance of genotypes with certainty, however significant environment suggests the effect of environment on particular genotypes which could be predicted with certainty. The magnitude of season effect was higher than the effect of genotypes and interaction for iron concentration. The contribution of environement component accounts for major portion of variability next to genotype. In maize, Oikeh et al. (2003), Boreddy et al. (2020) also explained significant G × E interaction effects (p < 0.05) for kernel iron but the environment effect was not significant. On the contrary the G × E interaction had contributed double the variance of genotypic component for kernel iron in maize. Presences of significant environmental and G × E interaction were also reported in other cereal crops. Mohammadi et al. (2015) reported winter rainfall and plant height to be the environmental and genotypic covariables, respectively, which contributed the

most to GE interaction for grain yield in rainfed durum wheat. Enyew *et al.* (2021) also reported variance due to genotype, environment and G×E interaction was highly significant for all traits in sorghum.

Transgressive Segregants: Improving nutrient content and yield potential is the prime objective of any crop breeding programme. Out of 120 RILs evaluated in the present study, 1 line and 3 lines were having iron content more than paternal parent CML 296 (>33.77 mg/kg) for *Rabi*, 2018 and *Spring*, 2019 respectively. During *Rabi*' 2018 season, out of 120 RILs studied, 39 lines were found to be early flowering and early maturing compared to parents. Early flowering and maturing maize cultivar are more remunerative and less risky. A total of 90 lines were with plant height lesser than both the parents (<128cm) and it offers scope for developing dwarf inbreds in maize. With regards to number of cobs per plant (25 lines with >1 cob/plant), cob weight (85 lines with >101 g), cob length (17 lines with >15.2 cm), cob width (120 lines

with >8.5 cm), number of kernels per row (1 line with >27), 1000 kernel weight (30 lines with>223g), yield per plot (15 lines with >1.89 kg) outperformed the parents. During Spring 2019 season, out of 120 RILs studied, about 46 lines were early flowering and early maturing as compared to parents. Around 72 lines were with plant height less than both the parents (<128cm) and this exhibit possibility to develop dwarf inbred in maize. The traits such as number of cobs per plant (22 lines with >1 cob/plant), cob weight (67 lines with >101 g), cob length (14 lines with >15.2 cm), cob width (120 lines with >8.5 cm), 1000 kernel weight (41 lines with >223g), yield per plot (18 lines with >1.89 kg) outperformed the parents. Sulewkska et al. (2008) found transgressive segregation in F2 generation of maize hybrids grown for grain and silage, according to them the yield of raw material for ensiling was lower from F2 generation. These traits have direct contribution towards the nutrient enrichment and yield improvement of maize. Thus, there is possibility for development of lines with better yielding potential than parental lines.

In the present study, phenotypic observations on iron content and agronomic traits revealed presence of sufficient amount of variability among RILs developed from the cross GLY6 ×CML296. The RILs with better mean values for iron content along with yield attributing traits will be useful to develop bio-fortified inbreds and subsequently for synthesising synthetics and single cross hybrids in maize. Recombinant inbred lines of maize can be used as mapping population for finding QTLs and mining out candidate genes for particular traits. Thus, the above study unravelled that information about the heritability, extent of variability and expected genetic advance for traits in the studied population which is essential for any crop improvement programme.

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