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A Study on Variability in Seed Morphology in *Shrunken*-2 Parental Lines and Hybrids of Sweet Corn

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ABSTRACT: A study was conducted to see the variability in sweet corn seeds size and shape and its correlation with field emergence. Grain size and shape properties were used for morphological variability assessment in the parental lines and hybrids of sweet corn. Seed's geometric features such as area, bounding box length and breadth, perimeter, axial length and width, median length and width, equivalent diameter, eccentricity and roundness were computed on the binary image with help of 'Grain Size and Shape Analysis' software. The results showed a considerable variability among genotypes for seed morphological characters. The study conducted a correlation heat-map analysis using various seed morphological characters and emergence percentage of seeds. The results revealed strong positive correlations among the seed morphological characters. The highest positive correlation was observed between the seed area and seed equivalent diameter (r = 0.99), as well as between the seed bounding box length and seed eccentricity (r = 0.99). The emergence percentage of sweet corn genotypes also exhibited correlations with the seed morphological characters. Among all the seed morphological characters, seed bounding box breadth showed the highest positive correlation (r = 0.57) with seedling emergence. The seed morphological parameters, found correlated with field emergence can be analysed and utilised to assess the performance behaviour of the sweet corn seeds.

Keywords: *Zea mays* L. var *saccharata*, seed morphology, emergence percentage, 'Grain Size & Shape Analysis', correlation heat-map.

INTRODUCTION

Maize (*Zea mays* L.) is the most important cereal crop in India after rice and wheat. It belongs to the *Maydeae* tribe and falls in the grass family *Poaceae*, having a chromosome number of 2n=20. This highly nutritious cereal is cultivated across an area of 193.7 million hectares worldwide, resulting in a substantial production of 1147.7 million tonnes and a productivity of 5.92 t/ha (FAOSTAT, 2020). Globally, maize has established itself as an industrial crop, with various applications. The majority of its production is utilized as animal feed (61%), while a significant portion is allocated for human consumption (17%), as well as in starch and processing industries (22%). Maize cultivation spans across 170 countries globally, with the leading maize producers being the USA, China, and Brazil. In India, maize is cultivated across 9.86 million hectares, resulting in an average production of 31.51 million tonnes and a productivity of 3.1 t/ha (DA & FW 2020-21). Approximately 47% of the maize produced in India is primarily used as poultry feed, with an additional 14% allocated for the starch industry. Furthermore, 13% serves as feed for livestock and food purposes, 12% used in various industrial sectors, 7% is processed for food consumption, and 6% is exported or utilized for other purposes.

Maize genotypes that possess kernels with enhanced biological and economic value are commonly known as "specialty corn". Among these, sweet corn (*Zea mays* L. var *saccharata*) is notable specialty maize characterized by its high sugar content in the kernels. Sweet corn is extensively utilized for human consumption and is a significant source of fiber, minerals, and vitamins (Chhabra *et al*., 2019). The primary factors contributing to its eating quality are flavour (particularly sweetness), kernel texture, and aroma (Evensen and Boyer 1986; Azanza *et al*., 1994). In sweet corn cultivation, several endosperm mutants, such as *shrunken* 2, *brittle* 1, *sugary* 1, and *sugary enhancer*, are employed to increase the sugar content in the kernels. Among these mutants, the *shrunken* 2 (*sh*2) variant exhibits the highest sucrose content (29.9%) and offers better storage quality for fresh cobs, making it extensively utilized in sweet corn breeding programs (Mehta *et al*., 2017).

However, despite these advantageous features, the commercial acceptance and widespread use of *sh*2 hybrids are limited due to challenges related to low seedling vigour, field emergence, and inconsistent stand establishment (Douglass *et al.*, 1993). Due to the higher sugar content in *sh*2*sh*2 mutants, the kernels have lower carbohydrate levels at seed maturity, leading to collapse and a shrunken appearance with varying degrees of opaqueness (Creech, 1965; Gubbels, 1975). The composition of maize kernels significantly affects seed vigour, longevity, and storage behaviour. Sweet corn varieties often exhibit poor seed germination and vigour due to insufficient nutrient supply during germination, attributed to low starch concentration, higher imbibition rates resulting in severe solute leakage, and susceptibility to physical damage and biotic stresses (Styer and Cantliffe 1983).

The concentration of sucrose in kernels is regulated by endosperm carbohydrate metabolism throughout their development (Kamol and Pulam 2007). *Sh*2 mutants exhibit higher levels of kernel sucrose, up to two to three times more, compared to conventional varieties, and they also retain higher sugar and moisture content for longer periods after harvest (Malvar *et al*., 1997). The timing of harvest plays a vital role in maintaining the desired quality of sweet corn kernels. Fully mature sweet corn tends to have larger kernels, while less mature kernels are smaller in size. Seed size and shape are generally associated with the seed potential in various crops. In the case of super sweet corn, research conducted by George *et al*. (2003) has shown a positive correlation between seed size and germination percentage in super-sweet corn hybrids. Catao *et al*. (2017) also used Groundeye software to assess the seedlings images through their hypocotyl and root lengths and seedling uniformity and growth. Furthermore, the Groundeye software has proven to be effective in evaluating the quality of popcorn seedlings and seeds that have been exposed to low temperature conditions.

In India, sweet corn cultivation is predominantly carried out during the winter season. However, this poses challenges to field emergence and overall crop establishment due to low temperatures (<10°C) experienced during this period (Parera *et al*., 1995). These cold temperatures act as a major bottleneck for sweet corn cultivation in farmers' fields. Keeping in view the above-mentioned background the present study was undertaken to see the variability in sweet corn seeds size and shape and its correlation with field emergence.

MATERIALS AND METHODS

A study was undertaken to evaluate the seedling emergence percentage under low temperature in *shrunken*2 based parental lines and hybrids of Sweet corn. The experiment was conducted at ICAR-Indian Agricultural Research Institute, (IARI) situated at 28°35′N latitude and 77°12′E longitudes and at an altitude of 228.6 m above mean sea level. It has a semi-arid and subtropical climate characterized with extreme hot summer and cool winter.

Research team at ICAR-Indian Agricultural Research Institute (IARI), New Delhi has developed a series of *sh2* based sweet corn inbred which are being used as parental lines for hybrid development. ICAR-IARI has recently released Pusa Super Sweet Corn-2 for hilly regions of India and identified another experimental hybrid which will be released soon. ICAR-VPKAS, Almora has also released a sweet corn hybrid; CMVL Sweet corn-1 for cultivation in hilly areas. The study was conducted with the parental lines and hybrids of public bred sweet corn hybrids; CMVL sweet corn-1and Pusa Super Sweet corn-2.

Female	Male	Hybrid
VSL16	VSL4	CMVL sweet corn-1
SWT16	SWT17	Pusa Super Sweet corn-2

The seeds of parental lines and hybrids of CMVL sweet corn-1 (VSL16/ VSL4) and Pusa Super Sweet corn-2 (SWT16/ SWT17) were procured from Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora and Maize section, Division of Genetics, ICAR-IARI, New Delhi.

Seed morphology assessment. A flatbed scanner (Cannon LiDE 110 version 1.2.00) was used for image acquisition. Grain size and shape properties were used for morphological variability assessment in the parental lines and hybrids of sweet corn. Seeds were placed on the flatbed scanner and geometric features such as area, bounding box length and breadth, perimeter, axial length and width, median length and width, equivalent diameter, eccentricity, and roundness were computed on the binary image with help of 'Grain Size and Shape Analysis' software.

Field emergence. Sweet corn seeds were sown in the field of Division of Seed Science and Technology on 20 February, 2020 and seedling emergence was observed up to 30 days. 100 seeds (25 seeds per row) each of parental lines and hybrids were planted in rows. Number of seedlings emerged in rows of each replicate were counted in each plot up to 30 days after sowing (DAS). Plate 1 shows emerged seedlings of parental line (VSL4) (a) and hybrid (CMVL Sweetcorn-1) (b) under field conditions. The parameter Emergence Percentage (EP) was calculated based on field emergence data. EP= (No. of normal seedlings on final count / total seed sown) × 100.

(a)

(b)

Plate 1: Field emergence of Parental line (VSL4) (a) and Hybrid (CMVL Sweetcorn-1) (b) under field conditions.

RESULTS

A flatbed scanner was used for image acquisition for morphological variability assessment in all the parental lines and hybrids of sweet corn. The following geometric features were computed on the binary image with help of 'Grain size and shape analysis' software.

Area of seed. The data recorded on area of sweet corn seeds is presented in Table 1. Among sweet corn genotypes studied, CMVL sweet corn-1, VSL16 and Pusa Super Sweetcorn-2 (45.04,43.52 and 43.41mm^2 respectively) had highest seed area and were statistically at par whereas, the lowest area was measured in parental line; SWT 16 (35.22mm^2).

Bounding box length. Bounding box length is the vertical coordinates of a rectangular border that encloses a digital image fully and measures the length of seed. The data recorded on bounding box length of sweet corn seeds showed maximum bounding box lengthinVSL4 (10.51mm) followed by CMVL sweet corn-1 (9.48 mm) and VSL16 (9.38mm) which were at par. The minimum bounding box length was measured in parental line; SWT 16 (7.39mm) (Table 1).

Bounding box breadth. Bounding box breadth is the horizontal coordinates of a rectangular border that encloses a digital image fully. Observations on the bounding box breadth of sweet corn seeds showed highest bounding box breadth in Pusa Super Sweetcorn-2, SWT 17, CMVL sweet corn-1 and VSL16 (6.86, 6.77, 6.63 and 6.58 mm respectively) which were at par statistically whereas the lowest bounding box breadth was measured in genotype; VSL4 (5.71mm) (Table 1).

Eccentricity. The deviation of a curve or orbit from the circularity is called eccentricity. Here in case of seed eccentricity indicates how the seed shape is not in round shape. The data recorded on eccentricity of sweet corn seeds expressed maximum eccentricity in VSL4 (0.85) followed by VSL16 and CMVL sweet corn-1 0.72 and 0.71 respectively) which were at par. The minimum eccentricity was observed in genotype SWT 16 (0.48) (Table 1).

Perimeter. The continuous line forming the boundary of a closed geometrical figure is known as perimeter. The perimeter of sweet corn seeds showed highest perimeter in VSL4 (28.28mm) followed by CMVL sweet corn-1 and VSL16 (27.30mm and 27.10mm) which were at par. The lowest perimeter was found in genotype; SWT 16 (22.95mm) (Table 1).

Table 1: Seed morphological characters of sweet corn genotypes.

Genotype/ Trait	Area(mm^2)	Bounding box Length(mm)	Bounding box Breadth(mm)	Eccentri-city	Peri-meter (mm)	Equivalent Diameter(mm)	Round-ness	Axial Length (mm)	Axial Width (mm)	Median Length (mm)	Median Width(mm)
VSL16	43.52	9.38	6.58	0.72	27.10	7.43	0.63	7.57	6.41	4.80	4.83
VSL4	41.57	10.51	5.71	0.85	28.28	7.25	0.48	7.93	5.57	4.85	4.17
CMVL SC-1	45.04	9.48	6.63	0.71	27.30	7.56	0.64	7.50	6.50	4.56	4.89
SWT 16	35.22	7.39	6.40	0.48	22.95	6.66	0.82	7.51	6.24	4.45	4.64
SWT 17	41.55	8.49	6.77	0.57	25.72	7.25	0.73	7.56	6.74	4.94	4.83
Pusa Super SC-2	43.41	8.92	6.86	0.62	26.61	7.40	0.70	8.43	6.63	5.47	4.69
Mean	41.72	9.02	6.52	0.66	26.32	7.26	0.66	7.75	6.35	4.85	4.67
C.D.	3.125	0.454	0.356	0.077	1.002	0.263	0.059	NS	0.33	NS	0.377
SE(m)	1.003	0.146	0.114	0.025	0.322	0.084	0.019	0.293	0.106	0.273	0.121

Equivalent diameter (mm). Equivalent diameter is the diameter of a hypothetical sphere composed of material having the same specific gravity as that of the actual soil particle and of such size that will settle in a given liquid at the same terminal velocity as the actual soil particle. Equivalent diameter indicates seed morphology. Highest equivalent diameter was measured in CMVL sweet corn-1, VSL16 and Pusa Super Sweetcorn-2 (7.56, 7.43 and 7.40 mm respectively) which were statistically at par whereas the lowest equivalent diameter was measured in SWT 16 (6.66mm) (Table 1).

Roundness. Roundness is the measure of how closely the shape of a seed approaches that of a circle. The data recorded on roundness of sweet corn seeds is depicted in Table 1. The sweet corn line; SWT 16 (0.82) had highest roundness followed by SWT 17 and Pusa Super Sweet corn-2 (0.73 and 0.70 respectively) which were at par. The lowest roundness was found in parental line; VSL4 (0.48).

Axial length. The distance between the anterior and posterior poles is the axial length. The axial length of sweet corn genotypes was not statistically different for this character that means all the genotypes were having more or less equal axial length of seeds (Table 1).

Axial width. The distance between the two horizontal poles of an image of seed is axial width. The axial width of SWT 17, Pusa Super Sweetcorn-2, CMVL sweet corn-1 and VSL16(6.74, 6.63, 6.50 and 6.41 mm respectively) were highest and statistically at par and the lowest axial width was measured in VSL4 (5.57mm) (Table 1).

Median length. The median length is the maximum length of a straight line connected by two points on the perimeter of a seed. The median length of sweet corn genotypes was not statistically different with respect to the trait, median length that means all the genotypes were having more or less equal median length of seeds (Table 1).

Median width. Medium width is the maximum length of a straight line perpendicular to the line of median length. Observations on the median width of sweet corn seeds showed minimum median width inVSL4 (4.17mm) and all the other genotypes under study were at par with respect to median width (Table 1).

Emergence percentage. The sweet corn genotypes were sown under sub optimum conditions in the field and their field emergence was observed (Plate 1). The hybrids outperformed parental lines for field emergence. The field emergence was highest in Pusa Super Sweetcorn-2 and CMVL sweet corn-1 which were at par (81 and 79 % respectively). The lowest (70 %) emergence percentage was observed in SWT17 (Fig.1). A significant difference was observed among the sweet corn genotypes for emergence percentage.

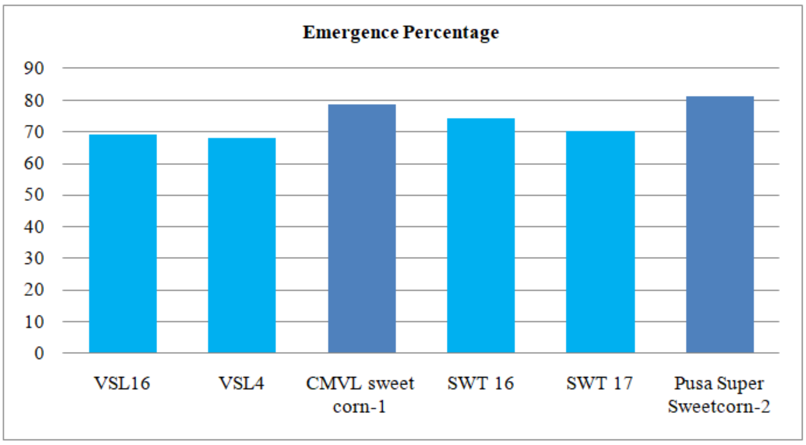

Fig. 1. Field emergence percentage of sweet corn genotypes under sub optimum field conditions.

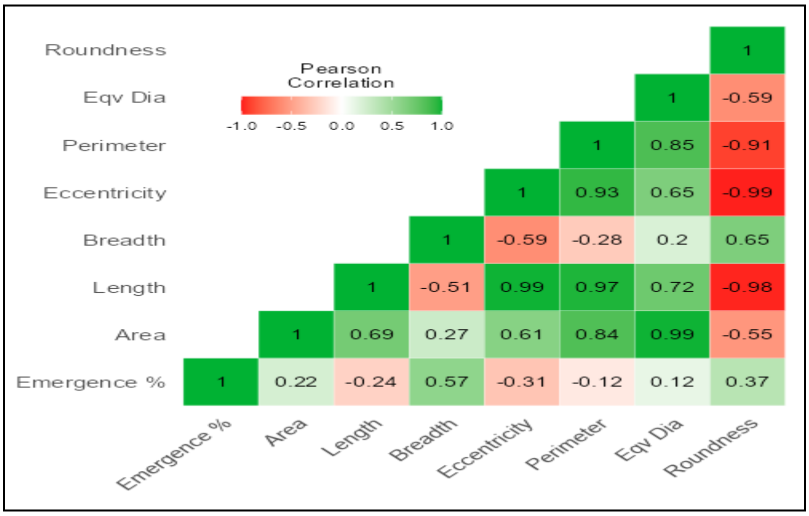

Fig. 2. Pearson correlation heat-map of seed morphological characters and emergence percentage of sweet corn genotypes.

Correlation Study. A correlation heat-map was constructed using emergence percentage, seed area, bounding box length and breadth, eccentricity, perimeter, equivalent diameter and roundness of seed. Seed morphological characters showed a highly positive correlation among themselves (Fig. 2). The highest positive correlation was observed among area of seed and seed equivalent diameter (r = 0.99); and seed bounding box length and seed eccentricity (r = 0.99) whereas, the highest negative correlation was observed among roundness of seed and seed eccentricity (r = -0.99) and seed bounding box length (r = -0.98). Emergence percentage of sweet corn genotypes was found correlated with seed morphological characters of sweet corn. Among all the seed morphological characters seed bounding box breadth had the highest positive correlation (r = 0.57) followed by seed roundness (r = 0.37) whereas, seed eccentricity and seed bounding box length were negatively correlated (r = -0.31 and r = -0.24) with seedling emergence.

DISCUSSION

Sweet corn is frequently subjected to cold stress and exhibits low seedling vigour, delayed emergence and poor stand establishment. Thus, there is a need to evaluate seed germination and vigour potential of sweet corn genotypes under suboptimum conditions for successful stand establishment in winter season. Therefore, the investigation was undertaken to study the seed germination status of sweet corn under low temperatures and its correlation with seed morphology. Seed size and shape is generally correlated with seed potential in several crops. In super sweet corn seeds, size was positively correlated with germination percentage by George *et al.* (2003) in super-sweet corn hybrids. The Groundeye software effectively assessed the quality of popcorn seedlings and seed subjected to low temperatures regimes. Catao *et al*. (2017) used Groundeye software to assess the seedlings images through their hypocotyl and root lengths and seedling uniformity and growth. Seed vigour imaging system was practical and valuable approach for evaluating the efficiency of priming treatment in sweet corn seeds using vigour index ratio of 70% growth index and 30% uniformity index for evaluating seed lots (Gomes *et al*., 2009). Seedling root length analysis using image analysis provided consistent data as compared to traditional vigour tests and it could be an efficient and time saving method for assessment of maize seed vigour (Dias *et al.,* 2015).

Seed size and shape is generally correlated with seed potential in several crops. Various softwares are available for assessment of seed quality in maize, among them, Groundeye software is being used to assess seedling uniformity and growth based on seedling images (hypocotyl and root length) (Catao *et al*., 2017). In our study, sweet corn genotypes assessed with 'Grain Size and Shape Analysis Software', differed for shape and size dimensions wherein hybrids had bolder seeds as compared to their parental lines. Thus, validating the better performance of hybrids over parental lines of sweet corn. Sulewska *et al.,* (2014) ; El-Abady, (2015) also reported higher seed size to be correlated with higher seed germination and vigour indices.

CONCLUSIONS

The present study was conducted to see the variability in sweet corn seeds size and shape and its correlation with field emergence. Seed's morphological characters such as area, bounding box length and breadth, perimeter, axial length and width, median length and width, equivalent diameter, eccentricity and roundness were having a considerable variability among genotypes. The Pearson correlation heat-map analysis done using various seed morphological characters and emergence percentage of seeds revealed a strong correlation among the seed morphological characters. The emergence percentage of sweet corn genotypes also exhibited correlations with the seed morphological characters. Thus, the seed morphological parameters, found correlated with field emergence can be analysed and utilised to assess the performance behaviour of the sweet corn seeds.

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Conflict of Interest. None.

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