



Physio-Morphological Characterization of Interspecific Hybridization-Derived Hull-Less Seeded Lines for Fruit and Seed Traits in Pumpkin

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Abstract Hull-less seed trait is readily available in *Cucurbita pepo*, but to enhance its yield in diverse climatic conditions transfer of this trait in another related species, viz. *C. moschata*, is highly desirable. Consequently, a set of total 33 hull-less seeded genotypes including 31 derived from interspecific hybridization between *C. pepo* (hull-less) and *C. moschata* (hulled), and two *C. pepo* genotypes were evaluated for various fruit and seed traits. Analysis of variance revealed significant variation among the genotypes for fruit (fruit weight, polar and equatorial diameter) and seed (seed length, width, thickness, number of seeds, and 100-seed weight) traits. An interspecific hybridization-derived genotype, viz. HL3643-6, was comparable to check *C. pepo* genotypes for most of the traits. All studied traits exhibited high heritability and genetic advance, with a significant positive correlation confirming their interrelationship. Principal component analysis indicated that the first two components contributed to 82.66% of the cumulative variance, with all eight traits contributing to PC1 and seed thickness along with hundred-seed weight being the major contributors to PC2. Overall, the investigation highlighted substantial variation in the interspecific derived lines and their potential for exploitation in improving the hull-less seed trait.

Keywords Correlation · Heritability · Hull-less seed · Principal component analysis · Variation

Introduction

The genus *Cucurbita* ($2n = 2 \times = 40$) is native to Americas and has a wide variability for vegetative, flowering, fruit and seed characteristics [9, 26]. Earlier the genus was considered to have 27 species; however, many of these species are highly cross-compatible [23]. Among the five (*C. pepo*, *C. moschata*, *C. maxima*, *C. ficifolia*, *C. argyrosperma*) domesticated species of *Cucurbita*, two, viz. *C. moschata* and *C. pepo* (pumpkins and squashes), are most cultivated and economically important [19]. The pumpkin

fruits are used for culinary purposes and have important roles in human health, blood purification, digestion and supplying energy [12]. Along with fruits, pumpkin seeds are also equally important due to their nutritional and pharmaceutical benefits and have 35–45% oil rich in health enhancing fatty acids like oleic, stearic, palmitic and linoleic acid [10].

A spontaneous mutation in pumpkin observed during 1880's in Austria, restricted the growth of seed coat layers and led to development of hull-less seeds (without seed coat) [17]. These seeds are important for snack and baking industries as they do not require the expensive decortication process [7]. However, the breeding programmes in *Cucurbita* have primarily focused on the development of varieties/hybrids with high fruit yield, improved quality, wide adaptability, and resistance to various biotic and abiotic stresses [11]. In contrary, the all-important pumpkin seeds have accorded little attention, but increasing people's awareness towards healthy food has led to improvement in

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breeding programmes for development of hull-less seeded varieties. But the complex nature of fruit and seed traits requires understanding of factors contributing to genetic variability present in the germplasm per se for the trait and interaction among them [16]. This will govern the selection criteria to be employed in the identification of promising genotypes with desirable traits. The estimates of heritability and genetic advance for the traits under improvement further guide in selection of superior genotypes as they measure the transmission of characters from one generation to another. Similarly, correlation analysis determines the association among traits and helps in designing selection strategies in the simultaneous improvement of a combination of traits. Concomitantly, assessment of genetic diversity in the germplasm helps in the identification of superior recombinants, where principal component analysis (PCA) helps in the identification of a set of genotypes capturing maximum genetic diversity [24].

In pumpkin, hull-less seed trait has been genetically mapped in *C. pepo* [14, 20]. In India, a cultivar, viz. 'PAU Magaz Kadoo-1,' in *C. pepo* genetic background has been released for commercial cultivation [6]. However, the trait is not available in *C. moschata*, thus interspecific hybridizations were performed [13], and presently, advanced generations of *C. pepo* × *C. moschata* are available with Punjab Agricultural University (PAU), Ludhiana. In view of the importance of fruit and seed traits in breeding for improved high seed yielding pumpkin varieties, an investigation was undertaken to evaluate and identify promising hull-less seeded genotypes.

Materials and Methods

Plant Material

Thirty-one hull-less seeded pumpkin genotypes (F_5 generation) derived from interspecific crosses of *C. pepo* (HLP-36 and HLP-639) with *C. moschata* (PVR-1343) were used in the study (Supplementary Table 1). Two locally adapted hull-less seeded *C. pepo* genotypes, viz. LGR-121 and PAU Magaz Kadoo-1 (PMK-1), were also included in the investigation as the check genotypes.

Analysis of Morpho-Physiological Traits

In summer 2021, seeds of all 33 genotypes were sown in plug trays and the germinated healthy seedlings were transplanted on both sides of beds, spaced 1.5 m (between beds) × 60 cm (within plants) at the Vegetable Experimental Field, PAU, Ludhiana, India. The plants were grown in randomized complete block design with three replicates for each genotype and ten plants per replicate.

The plants were allowed to open pollinate, and matured fruits were harvested 50–55 days after fruit setting. To evaluate phenotypic diversity, eight morphological descriptors, viz. fruit weight (FW, g), fruit polar diameter (FPD, cm), fruit equatorial diameter (FED, cm), seed length (SL, mm), seed width (SW, mm), seed thickness (ST, mm), number of seeds per fruit (NS) and 100 seed weight (100SW, g), were selected. Five fruits per replicate were harvested randomly for each genotype for data recording and statistical analysis.

The analysis of variance (ANOVA) was carried out using SPSS Software version 22 (IBM, Corp). The genetic variability estimates [Genotypic coefficient of variability (GCV), phenotypic coefficient of variability (PCV), broad sense heritability (Hb) and genetic advance as per cent mean (GA)] were obtained according to the methods suggested by Sivasubramanian and Madhavamenon [27] using the statistical software TNAU stat [21]. The correlation and principal component analysis were carried out using R packages 'corrplot' [29] and 'factoextra' [15], respectively.

Results

Analysis of Variance and Performance of the Genotypes

The analysis of variance (Supplementary Table 2) revealed significant differences for all the fruit and seed traits among the genotypes (Supplementary Fig. 1 and 2). The values of FW among the genotypes ranged from 91.46 to 1071.57 g with maximum in genotype HL3643-6 which was significantly better than both check genotypes, i.e. PMK-1 (569.29 g) and LGR-121 (535.28 g). FPD ranged from 3.37 (HL6772-1) to 12.04 cm (PMK-1), while FED varied from 4.47 (HL6772-1) to 11.95 (HL364367-7) cm. The genotype HL3643-6 was at par with check genotypes LGR-121 and PMK-1 for FPD, while for FED the genotypes HL364367-7 and HL3643-6 were at par with check PMK-1. Among the seed traits maximum SL and SW were observed in genotype HL3643-6 (13.67 and 8.86 mm), respectively, which was statistically similar with check genotype PMK-1. Seed thickness ranged from 1.11 (HL3643-1) to 2.0 mm (HL364343-4). The highest ST in interspecific hybridization-derived line HL364343-4 was statistically at par with the check genotypes; however, the maximum number of seeds per fruit were observed in PMK-1 (153.87) which was significantly better than all interspecific derived lines which could ultimately influence the seed yield. Hundred seed weight ranged from 3.19 to 8.71 g with maximum in HL3643-6 and lowest in HL6772-3. The hundred seed weight in HL3643-6 was significantly better than check genotypes (Supplementary Table 1).

Heritability Estimates

The estimates for components of variance, heritability and genetic advance are presented in Table 1. PCV estimates were slightly higher than the corresponding GCV for all the studied traits. The magnitude of PCV and GCV estimates was low for SL and SW, moderate for FED, NS and 100SW, while it was high for FW. The heritability estimates were moderate for ST and high for all other traits, while genetic advance (GA) was low for SL and ST, moderate for SW and FED and high for all other traits.

Correlation and Principal Component Analysis

Pearson's correlations between fruit and seed traits of hull-less seeded pumpkin genotypes are presented in Fig. 1 and Supplementary Table 3. There was significant positive association among the traits such as FW, FPD, FED, SL, SW, ST, NS and 100SW. The lowest but significant positive correlation was found between NS and ST. In the PCA, a total of eight principal components (PCs) were extracted and it revealed two most informative PCs with Eigen values of 5.81 and 0.80 which accounted for 82.66% of the cumulative variance (Supplementary Table 4 and Fig. 2A). In PC1, the rotated loading values ranged from 0.23 to 0.39, indicating approximately equal contribution towards the divergence. In PC2, the variation was accounted by ST (0.90) followed by 100SW (0.21), while all other traits did not substantiated for much variation (Fig. 2B). A biplot between PC1 and PC2 explaining 82.66% of variation demonstrated that all traits contributed almost equally to the PC1 while maximum contribution for PC2 was presented by ST. Three genotypes in PC1, viz. HL3643-6, HL364367-7 and PMK-1, and two genotypes in PC2, viz.

HL6772-1 and HL364343-1, contributed to the maximum genetic divergence (Fig. 2C).

Discussion

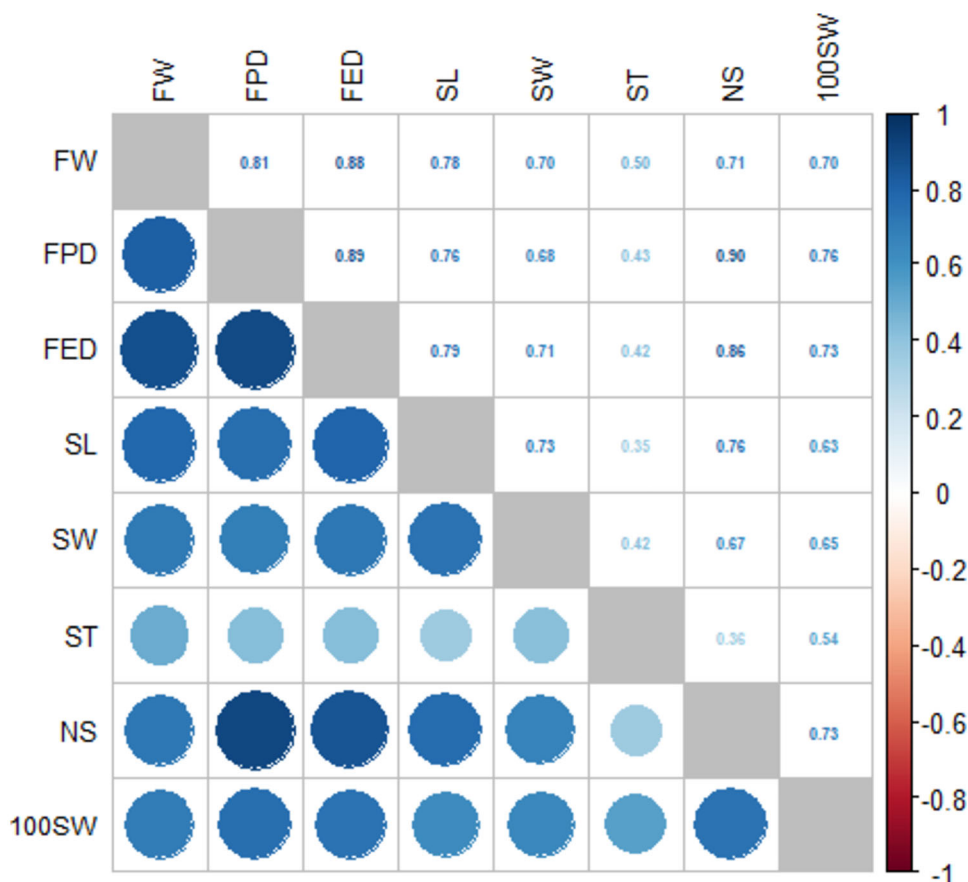
The genus *Cucurbita* comprises of wide variability, with *C. pepo* being most diverse followed by *C. moschata* [9, 25]. Fruit and seed traits are the common morpho-physiological traits accounting for the variability [5]. In this aspect, hull-less seed is an important quality trait that has primarily been reported with great variability in the *C. pepo* germplasm [17]. The biotechnological advancements have also been made for the hull-less seed trait in *C. pepo* where it has been linked to a lignin biosynthesis-related gene (Cp4.1LG12g04350) on chromosome 12 [14, 20, 22]. However, it has only recently been transferred to *C. moschata* through interspecific hybridization [13]. Therefore, a study was undertaken to evaluate the variability in the interspecific hybridization-derived hull-less seeded lines. The differences in the genetic makeup of the individual plants in a population constitute genetic variability and serve as a valuable source for selection of superior genotypes [4]. Significant genotypic differences were revealed with RBD and PCV and GCV estimates thereby suggesting that the genotypes can be used in further genetic analysis studies. The presence of significant differences among the genotypes in the current study is in consonance with Ezin et al. [8], wherein significant differences among pumpkin accessions were reported for fruit length, width and weight along with seed length, seed width, number of seeds and hundred seed weight. A wide range among the fruit and seed traits may also be attributed to the interspecific hybridization and selection of hull-less genotypes from F₂ generation irrespective of the horticultural traits.

Table 1 Estimates of components of variance, heritability (broad sense), GA and GA as per cent of the mean for fruit and seed traits of hull-less seeded pumpkin lines

Trait	General mean	PCV (%)	GCV (%)	Heritability (%)	GA	GA%
FW	501.76	51.81	50.05	93.31	499.73	99.60
FPD	7.16	34.73	33.51	93.10	4.77	66.60
FED	7.61	29.69	26.79	81.40	3.79	49.79
SL	11.52	13.14	10.19	60.10	1.87	16.27
SW	7.27	12.94	11.26	75.70	1.47	20.18
ST	1.64	17.75	12.47	49.34	0.30	18.04
NS	78.01	34.86	33.26	91.01	50.98	65.36
100SW	5.43	28.03	27.65	97.27	3.05	56.17

FW Fruit weight FPD Fruit polar diameter FED Fruit equatorial diameter SL Seed length SW Seed width ST Seed thickness NS Number of seeds 100SW 100 seed weight PCV Phenotypic coefficient of variation GCV Genotypic coefficient of variation GA Genetic advance GA% Genetic advance as per cent of mean

Fig. 1 Correlation coefficients of fruit and seed traits among hull-less seeded pumpkin lines; FW: Fruit weight; FPD: Fruit polar diameter; FED: Fruit equatorial diameter; SL: Seed length; SW: Seed width; ST: Seed thickness; NS: Number of seeds; 100SW: 100 seed weight



Similarly, a wide range for fruit and seed traits has been reported by Balkaya et al. [3] and Yadegari and Ansari [30]. A genotype, viz. HL3643-6, was found to be significantly better than the check genotypes for the most of the fruit and seed traits and hence can be exploited further for crop improvement programmes. Narrow range of differences in PCV and GCV estimates for all the traits implied minimal influence from environmental factors. Therefore, selection based on the phenotype can be effective for the improvement of these traits. Low differences between PCV and GCV observed for fruit weight, number of seeds and fruit diameter by Aruah et al. [2] corroborated with the current study. Further, as the estimates of GCV and PCV are high for the traits, direct selection for these traits would aid in genetic improvement. The contribution of genetic variability to the phenotypic variability is measured by heritability and works as an index for trait transmission from parents to their offspring. However, estimates of genetic advance along with heritability are more helpful in predicting the genetic gain under selection. Thus, selection of FW, FPD, NS and 100SW based on their high heritability and genetic advance estimates would be more effective as these traits are under the control of additive gene action and least influenced by the environment. These results of high heritability and genetic advance were in

agreement with the findings of Aruah et al. [2] and Ezin et al. [8].

Correlation studies are useful in understanding the association between fruit and seed traits, enabling plant breeders to select accessions possessing desirable traits related to seed yield. The correlations were significant and positive among the studied traits. Thus, the selection for the studied traits will also help in improving the seed yield. Similar to our finding, Aruah et al. [2] and Ezin et al. [8] also reported a significant positive correlation between fruit and seed traits. The principal component analysis attributed the variability to eight PCs. However, 88.66% of the total variance was explained from first two PCs; thus, selection of the traits that contributed through two major PCs would be beneficial. Similarly, two major PCs contributing to cumulative variance have been reported in cucumber [1]. The interspecific hybridization-derived genotypes, viz. HL3643-6 and HL364367-7, contributed maximum to the genetic diversity; hence, use of these accessions in the future hull-less seeded pumpkin improvement programmes will be beneficial [28]. Similar results of genetic variation in pumpkin were also reported by Balkaya et al. [3] and Liu et al. [18] with their genetic material. The divergence among hull-less seeded pumpkin genotypes is attributed to the diversity brought about by interspecific hybridization.

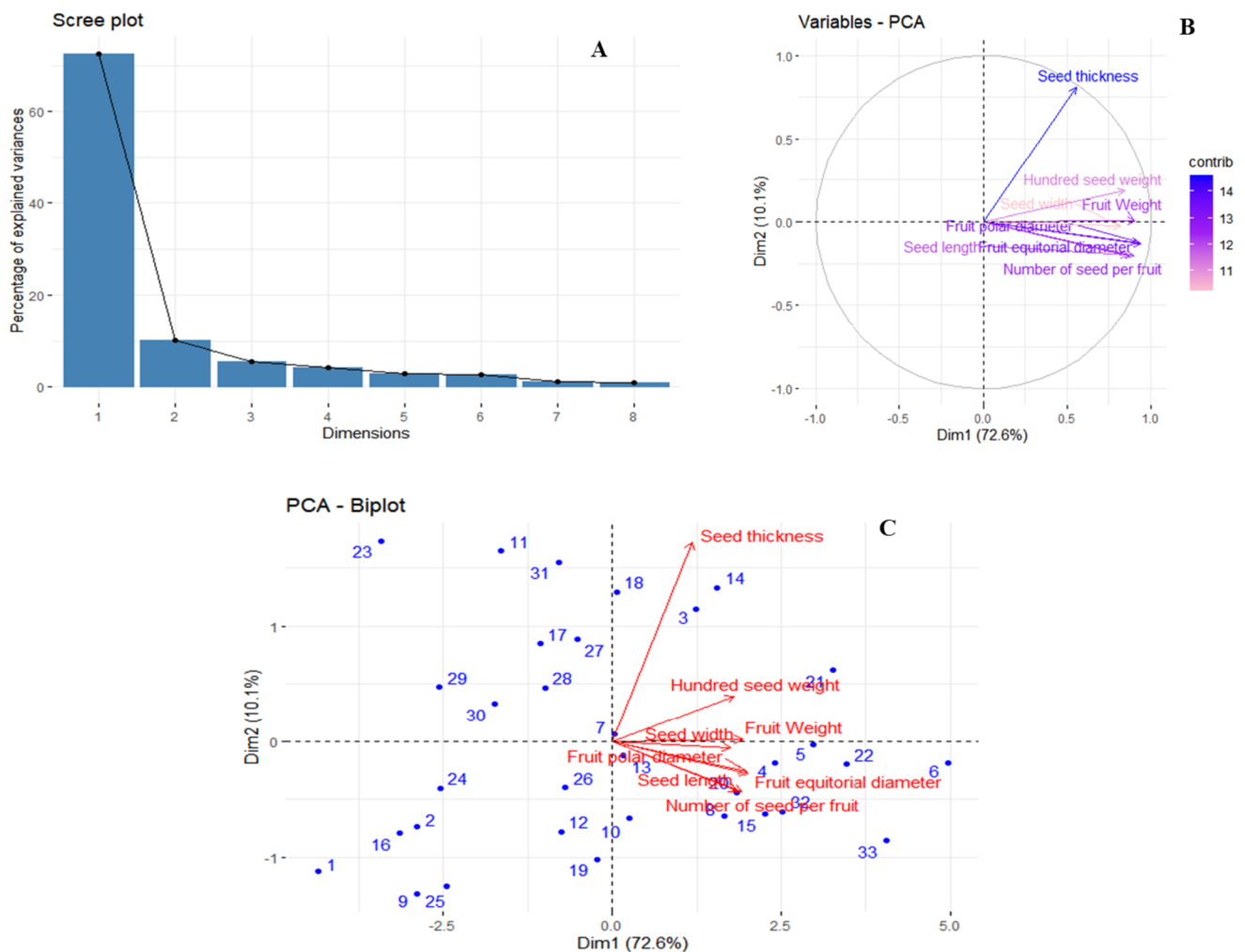


Fig. 2 **A** Scree Plot explaining the contribution of eight principal components, **B** PCA-Variables Plot, **C** PCA-Biplots explaining the contribution of eight traits to the total variation in the 33 hull-less seeded pumpkin lines

This diversification is valuable for developing commercially viable hull-less seeded pumpkin cultivars, which hold significant economic importance.

Conclusions

The assessment of fruit and seed traits in various inter-specific hybridization-derived hull-less seeded pumpkin genotypes revealed substantial variability. The traits such as fruit weight, polar and equatorial diameter, number of seeds, and 100-seed weight displayed high genetic variability and heritability, suggesting their suitability for direct selection in genetic improvement efforts. The positive correlations between fruit and seed traits underscored their association and signalled the potential for enhancing seed yield through trait selection. Principal component

analysis identified that all traits contributed to the overall variance. The promising traits demonstrated by genotypes, viz. HL3643-6 and HL364367-7, presented a potential for future pumpkin enhancement. Overall, the study underscores genetic variation's role in crop improvement, emphasizing the potential for further refinement.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s40003-024-00712-y>.

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Author's contribution ASD, MS, and JSK contributed to conceptualization and designing of the research work; KSG, NV, and BK contributed to execution of field experiments and data collection;

KSG, NV, BK, MS, JSK, and ASD contributed to analysis of data, interpretation and preparation of manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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