



Multivariate analysis techniques reveal significant morphogenetic variability in pumpkin landraces in Kenya

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Abstract

Documented information on naturalized pumpkin landraces in Kenya to identify useful variability is insufficient. The present study assessed variability using quantitative characters of 155 accessions, with 70 and 85 from Kakamega and Nyeri regions, respectively. The accessions were grown in one farm in a completely randomized design with three replications. Analysis of variance showed significant ($P < 0.05$) variation in all characters. Lowest and highest variables in mean and range were leaf length/width ratio and seed number that were 0.8 and 0.4, and 837 and 4,111, respectively. Eight factors accounted for 79.4% of total variation. The highly variable factors were fruit flesh thickness, length, width and length/width ratio, size, total weight, average weight and number, as well as seed number, 100-seeds weight, length, width, and thickness. Phenotypic coefficients of variation (PCV) were slightly higher or equal to genotypic ones (GCV). High GCV and PCV, heritability and genetic gain resulted for fruit size, total fruit weight, fruits and seeds. Over 70 positive correlations in fruit size, number and seeds with total fruit weight were observed in genotypic and phenotypic variabilities. Maximum positive direct effects on total fruit weight were observed in seeds, fruit average weight, length, and size, while indirect effects were observed in fruit number, flesh thickness, length, peduncle length and days to first flower. Multivariate analysis revealed fruit size, number, total weight, and seeds were characters of great genetic variability, which should be considered as primary components for achieving high yields in pumpkins when screening accessions for selection and improvement.

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Introduction

In Kenya, naturalized pumpkin accessions are considered rich sources of variation (Ragheb, 2016). However, documented information about them is insufficient to identify genetic variability and enable selection of desirable traits (Fayeun *et al.*, 2016). Nonetheless, introduced exotic cultivars are endangering the naturalized variability (Marilene *et al.*, 2012) that has already disappeared (Mohammed and Zakri, 2001) and the remaining few stands with farmers are now gathered with great drudgery. Therefore, germplasm collection (Kumar *et al.*, 2014) and evaluation of the amount and nature of morphogenetic variation in characters of economic interest is of critical importance (Fayeun *et al.*, 2012).

Genetic integrity of pumpkin accessions with desirable traits is assessed by grouping identifiable and measurable traits (IBPGR, 1991) into either qualitative observable or quantitative non-observable traits (Stalker and Chapman, 1989). Factor analysis classifies and describes variations, guides on the choice of parents for plant hybridization, and groups genotypes with one or more similar characters into clusters. It examines multiple measurements of multivariate data to identify fundamental and meaningful dimensions that explain the observed relationship of numerous plant traits (Odiyi *et al.*, 2014). Large datasets are reduced into factors that underlie the quality of characteristics of the original characters by regrouping them into a limited set of descriptive categories of fewer latent traits sharing common variance (Yong and Pearce, 2013). It assumes small unobserved (latent) traits are responsible for the correlation among a large number of observed and interdependent traits (Odiyi *et al.*, 2014), and summarizes the underlying variations into relationships and patterns that can be easily interpreted and understood to ensure only key factors are considered rather than focusing on too many trivial traits (Yong and Pearce, 2013).

Improvement of plants depends on the amount of variability (Reddy *et al.*, 2013, Sultana *et al.*, 2015), and heritability of desirable characters (Fayeun *et al.*,

2016) present in germplasm. Assessing genetic variability identifies genotypes with desirable traits (Riley *et al.*, 1996), and increases efficiency to improve and properly manage crop selections (Geleta *et al.*, 2005). Phenotypic and genotypic variability, broad sense heritability and genetic gain reveal the stability and sustainability of plant species to environmental conditions (Mohammed and Zakri, 2001; Grubben and Chigumira, 2004; Roychowdhury and Tah, 2011). Thus, partitioning genetic variability into heritable and non-heritable components is important for effective selection of plants with desirable traits (Fayeun *et al.*, 2016). Phenotypic and genotypic variance components estimate variation within and between germplasm (Maji and Shaibu, 2012). Genotypic and phenotypic coefficients of variation evaluate the relative contribution of various characters to total variation (Dilruba *et al.*, 2014) and represent the overall influence of a particular character on yield (Mahaveer *et al.*, 2017). They enable indirect selection of traits that are hard to select by another directly correlated trait of higher genetic gain and express the strength of association between all possible pairs of traits (Kraic *et al.*, 2009) to give additional information that can be used to discard or promote genotypes of interest (Machado *et al.*, 2017).

Path coefficient analysis provides an effective means for a critical examination of specific forces of action that produce a given correlation and measure the relative importance of each factor when two characters show correlation just because they are correlated with a common third one (Tiwari and Upadhyay, 2011). The contributions of various independent variables on a dependent variable (Mahaveer *et al.*, 2017; Sultana *et al.*, 2015) are partitioned by splitting the total correlation coefficients of different characters into direct and indirect effects on yield in such a manner that the sum of direct and indirect effects is equal to total genotypic correlation (Tiwari and Upadhyay, 2011). The derived information enables breeders to identify important component characters that can be utilized to improve yield (Mahaveer *et al.*, 2017). It also

provides interpretation of the relationships between and within the causal factors contributing to the observed effects (Akintunde, 2012).

Despite the aforementioned knowledge, no serious attempts have been made to assess the genetic variability of naturalized pumpkins in Kenya. Information available on inherent variability (Malashetty, 2010) that can be used for effective selection and improvement (Fayeun *et al.*, 2012) of naturalized pumpkin is scanty. The present study assessed genetic variability and interrelationships of characters and partitioned the contribution of characters into direct and indirect effects towards fruit yields of naturalized pumpkin accessions. More than one technique was used for validation purposes (Odiyi *et al.*, 2014). Considering the genetic erosion risk facing naturalized pumpkins (Marilene *et al.*, 2012), this paper presents valuable information for selection and improvement of pumpkins in Kenya.

Materials and methods

Research site

The 155 pumpkin accessions collected from smallholder farms in Kakamega and Nyeri regions were planted on 23rd May, 2012 in Chuka University research farm in a Completely Randomized Design (CRD) with three replications and 2 m x 2 m spacing. The farm lies at 0°19' S, 37°38' E and 1535 m above sea level. Annual precipitation is about 1,200 mm and is bimodally distributed, with long rains falling from March to June and short rains from October to December. Annual mean temperature is about 20°C. Soils are mainly humic nitisols, deep, well-weathered with moderate to high fertility (Jaetzold *et al.*, 2005).

Site preparation

Land was ploughed and pulverized to a fine tilth using a fork hoe. Planting holes measuring 60 cm² on top by 60 cm depth were excavated and the top soil separated from the subsoil. The top soil was mixed with 24 kg farm yard manure (FYM) and returned into each hole, leaving 15.2 cm unfilled portion. The holes were planted with five plants of each accession. Recommended pesticides were sprayed to control

insects and diseases. Moles were trapped and weeds removed manually. The plants were irrigated every week with 20 L of water per hole up to fruit maturity whenever rains failed.

Character evaluation

Data recording started 20 days after emergence and continued up to harvesting stage. Five plants per accession were selected and tagged for evaluation based on IPGRI descriptors (IPGRI, 2003). Data on leaves, stems and inflorescence were recorded on 146 accessions, and on fruits and seeds on 126 and 124 accessions, respectively. Main vine length was measured using a tape measure. Plant size (m³) assessment was done 30 days after germination, as: $L \times W \times H$, where: L = length (m) measured from the base to the furthest point on a main vine; W = width (m) measured at the widest section; and H = height (m) measured from the base of a plant to the highest point. Leaf size was estimated using leaflets in a leaf (Nt) combined with the length (Lc) and maximum width (Wc) of the central leaflet (Akoroda, 1993). Leaf Area = $0.9467 + 0.2475LcWc + 0.9724LcWcNt$ ($r^2 = 0.92$), where: Lc = length of central leaflet; Wc = maximum width of central leaflet; Nt = number of leaflets in a leaf; and r^2 = coefficient of determination (Fubara-Manuel *et al.*, 2012). The fruits were counted, weighed and averaged. Fruit size variability was derived from coefficient of variation = $(100 \times \text{standard deviation}) / \text{total mean weight of fruits}$ (Newsom *et al.*, 1993). Fruit length and width were assessed using a tape measure. Total and average seeds were counted for each accession. The 100-seed weight was determined using a 200 g electronic balance. Fruit flesh, stem and seed thickness were measured using a Vernier caliper.

Data analysis

The means of quantitative data values on five plants per accession per replication were subjected to analysis of variance using SAS program. The means and ranges were used to determine the extent of variability of each character. Factor analysis was performed using SPSS at $P = 0.05$ to assess the variability of accessions in relation to the most

discriminating character (Nwofia *et al.*, 2012). Characters with loads ≥ 0.50 were considered to be highly relevant in contributing to the total variation. Factors with Eigen values equal or greater than 2.0 were the only ones retained for interpretation purposes (Norman *et al.*, 2011).

Genotypic (σ^2g), phenotypic (σ^2p) and environmental (σ^2e) variances were used to estimate variability (Kwon and Torrie, 1964). Genotypic variance (σ^2g) = (MSA – MSE)/r; Phenotypic variance (σ^2p) = ($\sigma^2g + \sigma^2e$)/r; Environmental variance (σ^2e) = MSE, where MSA, MSE and r refer to mean squares of accessions, mean squares of error, and number of replications, respectively (Ahsan *et al.*, 2015). Phenotypic (PCV) and genotypic (GCV) coefficients of variation were calculated using the formula: PCV (%) = ($\sigma p/X$) *100, GCV (%) = ($\sigma g/X$)*100, where σp , σg and X shows phenotypic, genotypic standard deviations and grand mean for respective characters (Singh and Chaudhary, 1985; Bozokalfa *et al.*, 2010).

Broad sense heritability ($h^2B\%$) was estimated on genotypic mean using the formula: Heritability (h^2B) = σ^2g/σ^2p , where h^2B = broad sense heritability, σ^2g = genotypic variance, σ^2p = phenotypic variance (Bozokalfa *et al.*, 2010). Expected genetic advance (GA) and percentage of GA of the mean were calculated using the formula: Expected genetic advance (GA) = $i\sigma p h^2$; GA% = (GA/X) *100, where i = standardized selection differential constant 2.06 at $P=0.05$, σp = phenotypic standard deviation, h^2B = broad sense heritability, X = grand mean (Bozokalfa *et al.*, 2010).

Phenotypic and genotypic correlation coefficients were estimated using covariance components between pairs of characters (Kassahun *et al.*, 2013; Dilruba *et al.*, 2014) as:

$$r_{g_{xy}} = \frac{GCOV_{XY}}{\sqrt{\sigma_{g_x}^2 \cdot \sigma_{g_y}^2}} \quad \text{and} \quad r_{p_{xy}} = \frac{PCOV_{XY}}{\sqrt{\sigma_{p_x}^2 \cdot \sigma_{p_y}^2}}$$

Where: r_{gxy} = genotypic and r_{pxy} = phenotypic correlation coefficient between characters x and y,

$r_{p_{xy}}$ = phenotypic correlation coefficient between characters x and y, σ^2g_x and σ^2g_y = genotypic variance of characters x and y, σ^2p_x and σ^2p_y = phenotypic variance of character x and y, respectively. Phenotypic correlation coefficients significance were tested using tabulated 't' value at (a- 2) degrees of freedom, where 'a' is number of accessions; while genotypic correlation coefficients significance were tested using the formula: ,

$$t = \frac{r_{gxy}}{SE_{r_{gxy}}}, \quad SE_{r_{gxy}} = \sqrt{\frac{(1-r^2)^2}{2H_x \cdot H_y}}$$

Where: h^2x and h^2y are heritability for character x and y (Kassahun *et al.*, 2013). The calculated 't' value was compared with tabulated 't' at (a-2) degrees of freedom and $P=0.05$.

Path coefficient analysis was estimated on genotypic correlation coefficients with total fruit weight as the dependent variable and the other characters as independent variables. The direct effects on the dependent variable were estimated by regression analysis of standardized data of the characters using SPSS. The indirect effects of the independent

variables were estimated as:

$$r_{ij} = P_{ij} + \sum r_{ik} \times P_{kj}$$

Where: r_{ij} = mutual association between the independent character (i) and dependent character (j) as measured by genotypic correlation coefficient, p_{ij} = the component of direct effects of the independent character (i) on the dependent character (j) as measured by genotypic path coefficients, $r_{ik}p_{kj}$ = the summation of components of the indirect effect of a given (i) independent character on given (j) dependent character via all other (k) independent characters (Khan *et al.*, 2016). The residual effects (h)

were recalculated using the formula:

$$h = \sqrt{(1 - R^2)}$$

Where, R^2 is calculated as $\sum r_{ij}^2$ (Khan *et al.*, 2016).

Results and discussion

Morphological variability

Analysis of variance, range and mean values: All the

characters showed significant ($P < 0.05$) variations (Table 1).

Table 1. Minimum, maximum, range and mean values for each quantitative character.

SN	Quantitative trait	Accession No.	Min.	Max.	Range	Mean	P-value
1	Plant size (m ³)	146	0.6	6.6	6.0	2.6	0.00
2	Internode length (cm)	146	9.0	25.0	16.0	17.8	0.00
3	Number of nodes to first fruit	146	13.0	47.0	34.0	26.1	0.00
4	Stem thickness (mm)	146	7.9	14.9	7.0	10.7	0.00
5	Leaf size (cm ²)	146	36.0	70.5	34.5	49.7	0.00
6	Leaf length/width ratio	146	0.6	1.0	0.4	0.8	0.00
7	Days to first flowering	146	49.0	87.0	38.0	69.1	0.00
8	Peduncle length (cm)	126	4.0	16.5	12.5	8.3	0.00
9	Fruit flesh thickness (mm)	126	10.5	42.6	32.1	25.0	0.00
10	Fruit length (cm)	126	7.0	36.0	29.0	14.9	0.00
11	Fruit width (cm)	126	8.0	20.0	12.0	13.4	0.00
12	Fruit length/width ratio	126	0.5	3.0	2.5	1.2	0.00
13	Fruit size	126	0.4	76.3	75.9	16.6	0.00
14	Days to first fruiting	126	107.0	141.0	34.0	127.6	0.00
15	Maturation period	126	39.0	89.0	50.0	56.9	0.00
16	Total fruit weight/accession (kg)	126	0.3	19.3	19.0	3.9	0.00
17	Number of fruits/plant	126	1.0	13.0	12.0	3.4	0.00
18	Fruit weight/accession (kg)	126	0.3	3.0	2.7	1.2	0.00
19	Weight of 100 seeds (g)	124	6.3	27.1	20.8	12.4	0.00
20	Number of seeds/plant	124	40.0	4151	4111	837	0.00
21	Average seeds/fruit	124	33.0	611	578	242	0.00
22	Seed thickness (mm)	124	11.2	21.3	10.1	3.2	0.00
23	Seed length (mm)	124	7.0	13.1	6.1	15.4	0.00
24	Seed width (mm)	124	2.2	5.0	2.8	8.8	0.00
25	Seed length/width ratio	124	1.3	2.7	1.4	1.8	0.00

The magnitude of range was higher than the corresponding mean for most characters, but was lower than the corresponding mean for internode length, stem thickness, leaf size, length/width ratio, days to first flowering, days to first fruiting, fruit width, seed length, width, and length/width ratio.

The magnitude of range was more than double the corresponding mean for plant size, fruit size, length/width ratio, total, average weight, number, and seed number, average, and thickness per accession. The mean for each character showed considerable variations among the pumpkin accessions. The lowest and highest range and mean values were for leaf length to width ratio and seeds per pumpkin accession, respectively.

The significant variations observed in all the characters indicated existence of sufficient morphological variability and ample scope for selection (Parikh *et al.*, 2012; Oliveira *et al.*, 2016).

Aruah *et al.* (2010) and Srikanth *et al.* (2017) reported significant variations in most characters, while Fukrei *et al.* (2011) and Mladenovic *et al.* (2014) in all characters in pumpkins.

The variation in mean among the accessions for each character indicated existence of morphological variability (Fayeun *et al.*, 2012), suggesting that the accessions were distinct from each another, but they could have shared almost the same pattern of gene action (Ogunniyan and Olakojo, 2014).

Table 2. Factor components, Eigen values, loadings, communality and specificity of quantitative traits

Factor components	Factor1	Factor2	Factor3	Factor4	Communality	Specificity
Eigen values	5.0	3.7	2.6	2.2		
EPV(%)	20.2	14.7	10.6	8.8		
CPV(%)	20.2	34.8	45.5	54.2		
Characters	Loadings				%	%
Plant size (m ³)	0.05	0.14	0.07	-0.09	63.7	36.3
Internode length (mm)	-0.11	0.12	0.14	0.23	61.6	38.4
Number of nodes of first fruit	0.03	-0.11	0.12	0.02	59.7	40.3
Stem thickness (mm)	0.72	0.00	-0.18	-0.04	77.4	22.6
Leaf size (cm ²)	0.50	0.05	-0.05	0.00	63.5	36.5
Leaf length/width ratio	-0.25	0.01	-0.07	-0.01	60.7	39.3
Day to first flowering	-0.73	0.18	0.21	0.07	77.1	22.9
Peduncle length (cm)	-0.41	0.20	0.12	0.21	51.8	48.2
Fruit flesh thickness (mm)	-0.27	0.13	0.84	0.01	80.2	19.8
Fruit length (cm)	0.01	0.09	0.26	0.91	92.2	7.8
Fruit width (cm)	0.03	0.18	0.86	-0.28	89.5	10.5
Fruit length/width ratio	-0.01	-0.04	-0.21	0.94	93.2	6.8
Fruit size	-0.20	0.94	0.05	-0.05	95.8	4.2
Day to first mature fruit	0.10	-0.05	0.07	0.07	90.1	9.9
Maturation period	0.56	-0.18	-0.02	-0.01	89.5	10.5
Total fruit weight/accession (kg)	-0.15	0.85	0.40	0.15	93.7	6.3
Number of fruits/plant	-0.22	0.94	-0.05	-0.07	96.3	3.7
Fruit weight/accession (kg)	-0.03	0.01	0.82	0.37	85.2	14.8
Weight of 100 seeds (g)	0.83	-0.20	0.08	0.09	79.1	20.9
Number of seeds/plant	-0.18	0.90	0.10	0.08	94.0	6.0
Average seeds/fruit	-0.06	0.10	0.26	0.28	69.2	30.8
Seed length (mm)	0.88	-0.18	0.00	-0.09	87.1	12.9
Seed width (mm)	0.87	-0.17	-0.11	0.04	88.9	11.1
Seed thickness (mm)	0.81	-0.18	-0.09	0.04	81.4	18.6
Seed length/width ratio	-0.18	-0.05	0.06	-0.12	72.1	27.9

EPV=Explained proportion of variation; CPV=Cumulative proportion of variation.

Ragheb (2016) reported differences in mean for vine length and number of branches per plant in pumpkins. Character means are important when choosing crops as sources of inbred lines and hybrids (Hallauer *et al.*, 2010). Range values more than double the mean suggested existence of adequate morphological variability among accessions (Ogunniyan and Olakojo, 2014). Ragheb (2016) reported range values more than double the mean in particular characters of sweet melon and in most characters of cucumber. Small range values less than the mean were attributed to the practice by farmers of growing pumpkins for generations without any purification or improvement (Ragheb, 2016). Farmers

have also selected and recycled planting seeds every season over the years, exchanged seeds and traded fruits within and beyond borders. They have also maintained more than one distinct landrace as a variety, which they carry over from one generation to the next. This practice fixes and separates favourable genotypes of interest, reduces the percentage of heterozygotes and effective population size unknowingly, thereby increasing the opportunity for fixation of alleles (Ghebru *et al.*, 2002). Fixation of alleles increases homozygosity within genotypes (Ogunniyan and Olakojo, 2014), reduces cultivar fitness, increases genetic variance between and reduces it within families with progressive increase in

additive variance at the expense of dominance in totally homozygote lines (Grisales *et al.*, 2009). The observed significant variation provide a better chance for improvement, hence accession that achieve high

performance in quantitative economic characters should be selected for improving naturalized pumpkins (Hallauer *et al.*, 2010; Ragheb, 2016).

Table 3. Estimation of GV, PV, GCV%, PCV%, heritability and genetic gain of quantitative characters.

Character	Mean	σ^2_g	σ^2_p	σ^2_e	GCV%	PCV%	h^2_B	GA	GA%
Plant size (m ³)	2.55	1.70	1.70	0.00	51.16	51.16	100.0	2.69	105.39
Internode length (mm)	17.78	8.35	9.68	1.33	16.25	17.50	86.26	5.53	31.10
No. of nodes to first fruit	26.07	36.89	40.46	3.57	23.30	24.40	91.18	11.95	45.83
Stem thickness (mm)	10.65	2.03	2.16	0.13	13.38	13.81	93.98	2.85	26.73
Leaf size (cm ²)	49.69	35.90	39.20	3.30	12.06	12.60	91.58	11.81	23.77
Leaf length/width ratio	0.77	0.00	0.00	7.96	8.48	88.0	12.0	15.38	7.96
Days to first flowering	69.10	82.07	103.18	21.11	13.11	14.70	79.54	16.64	24.09
Peduncle length (cm)	8.29	5.47	5.54	0.07	28.21	28.39	98.74	4.79	57.75
Fruit flesh thickness (mm)	24.99	39.13	41.25	2.12	25.03	25.70	94.86	12.55	50.23
Fruit length (cm)	14.89	18.22	19.43	1.21	28.67	29.61	93.77	8.51	57.20
Fruit width (cm)	13.37	6.00	6.18	0.18	18.32	18.59	97.09	4.97	37.19
Fruit length/width ratio	1.16	0.16	0.17	0.01	34.54	35.61	94.12	0.80	69.04
Fruit size	16.62	192.59	192.59	0.00	83.49	83.49	100.0	28.59	171.99
Days to first fruiting	127.60	47.66	49.25	1.59	5.41	5.50	96.77	13.99	10.96
Maturation period	56.90	137.77	145.51	7.74	20.63	21.20	94.68	23.53	41.35
Total fruit weight/acc (kg)	3.92	8.28	8.28	0.00	73.43	73.43	100.0	5.93	151.27
Number of fruits/plant	3.41	5.03	5.03	0.00	65.72	65.72	100.0	4.62	135.38
Average fruit wt/acc (kg)	1.17	0.27	0.27	0.00	44.51	44.51	100.0	1.07	91.69
100-seed weight (g)	12.37	18.42	18.42	0.00	34.71	34.71	100.0	8.84	71.50
Number of seeds/plant	836.64	385376.4	385376.4	0.00	74.20	74.20	100.0	1278.8	152.85
Average seeds/fruit	242.29	10653.5	10653.5	0.00	42.60	42.60	100.0	212.6	87.76
Seed length (mm)	15.37	3.55	3.57	0.02	12.26	12.29	99.44	3.87	25.18
Seed width (mm)	8.76	1.63	1.64	0.01	14.57	14.61	99.39	2.62	29.92
Seed thickness (mm)	3.18	0.40	0.40	0.00	19.88	19.88	100.0	1.30	40.96
Seed length/width ratio	1.77	0.03	0.03	0.00	9.78	9.78	100.0	0.36	20.16

GV (σ^2_g) = Genotypic variance, PV (σ^2_p) = Phenotypic variance, EV (σ^2_e) = environmental variance, GCV% = Genotypic coefficients of variation percentage, PCV% = Phenotypic coefficients of variation percentage, h^2_B = broad sense heritability, GA = Genetic advance, GA% = Genetic advance percentage, acc = accession.

Tables 4 and 5 Key

Values in bold (Table 4) and as terix* (Table 5) are significantly different at $P=0.05$. PS = plant size, IL = internode length, NFF = number of nodes to the first flower, STT = stem thickness, LS = leaf size (cm²), LR = leaf length/width ratio, DFF = days to first flowering, PL = peduncle length (cm), FT = flesh thickness (mm), FL = fruit length (cm), FW = fruit width (cm), FR = fruit length/width ratio, FSV = fruit size variability, DFM = days to first mature fruit, MP = maturation period (days), TFW = total fruit weight/accession (kg), NFA = number of fruits/accession, AFW = average fruit weight/accession (kg), WHS = weight of 100 seeds, NSA = number of seeds/accession, ASF = average seeds/fruit, SL = seed length (mm), SW = seed width (mm), ST = seed thickness (mm), SR = seed length/width ratio, TFWCC = total fruit weight correlation coefficients.

Factor analysis: The 25 quantitative characters were grouped into 8 factors that explained 79.7% of total variation (Table 2). The first four factors explained 54.2% of total variation, where factor one had the

highest Eigen-value and accounted for the greatest amount of total variation. It was highly and positively loaded for stem thickness, leaf size, and fruit maturity period, 100-seed weight, seed length, width and

thickness, but highly and negatively loaded for daysto first flowering. Factor two was highly and positively loaded for fruit size, total weight, number and seeds per accession; factor three for fruit flesh thickness, width and average weight per accession; and factor four for fruit length and length/width ratio. The communalities were high over specificities in all the

characters. Most fruit and seed traits had communality values above 80%. Peduncle length had the lowest communality, while fruits per plant had the lowest specificity (Table 2). The degree of association within the first three factors was used to construct a three dimensional ordination that explained 45.5% total variation.

Table 4. Phenotypic (above) and genotypic (below) correlation coefficients of different quantitative characters ($P < 0.05$) among pumpkin accessions.

	PS	IL	NFF	ST	LS	LR	DFF	PL	FT	FL	FW	FR	FSV	DFM	MP	TFW	NFA	AFW	WHS	NSA	ASF	SL	SW	ST	SR
PS	1	0.31	0.12	0.18	0.28	0.00	0.05	0.04	0.13	0.04	0.20	-0.07	0.18	-0.05	0.02	0.20	0.16	0.09	-0.03	0.22	0.15	0.01	0.01	-0.02	0.03
IL	0.33	1	-0.08	0.04	0.12	0.00	0.07	0.14	0.10	0.19	0.10	0.09	0.18	-0.02	-0.04	0.25	0.18	0.14	-0.06	0.19	0.00	-0.03	-0.05	-0.09	-0.01
NFF	0.13	-0.09	1	0.06	0.14	0.00	0.13	0.09	0.04	0.00	0.02	0.00	-0.07	0.06	0.01	-0.02	-0.10	0.09	0.04	-0.07	0.00	-0.01	-0.01	0.03	0.04
ST	0.19	0.05	0.07	1	0.64	-0.02	-0.56	-0.33	-0.26	-0.02	-0.04	-0.01	-0.13	0.11	0.44	-0.13	-0.13	0.44	-0.09	-0.03	0.60	0.57	0.53	-0.08	
LS	0.30	0.14	0.15	0.69	1	-0.01	-0.31	-0.13	-0.13	0.03	0.05	0.00	-0.06	0.15	0.29	-0.03	-0.06	-0.02	0.29	0.03	0.08	0.39	0.35	0.29	-0.07
LR	0.00	0.00	0.00	-0.02	-0.01	1	0.03	0.02	0.00	0.00	0.00	0.00	0.00	0.00	-0.01	0.00	0.00	0.01	-0.01	0.00	0.00	-0.01	-0.01	-0.02	0.01
DFF	0.06	0.08	0.15	-0.65	-0.36	0.03	1	0.47	0.35	0.14	0.23	0.03	0.26	-0.13	-0.70	0.34	0.29	0.24	-0.50	0.29	0.19	-0.56	-0.63	-0.66	0.21
PL	0.04	0.15	0.09	-0.34	-0.14	0.02	0.53	1	0.29	0.24	0.16	0.13	0.20	-0.10	-0.39	0.34	0.25	0.27	-0.36	0.33	0.29	-0.30	-0.45	-0.47	0.31
FT	0.13	0.11	0.04	-0.28	-0.14	0.00	0.40	0.30	1	0.28	0.72	-0.08	0.20	-0.10	-0.25	0.49	0.19	0.63	-0.21	0.26	0.25	-0.26	-0.36	-0.33	0.14
FL	0.05	0.21	0.01	-0.02	0.03	0.00	0.16	0.25	0.30	1	0.11	0.79	0.04	0.08	-0.05	0.33	0.04	0.55	0.06	0.18	0.36	-0.07	-0.05	-0.01	0.00
FW	0.20	0.11	0.02	-0.04	0.05	0.00	0.26	0.17	0.75	0.12	1	-0.39	0.19	0.02	-0.10	0.46	0.18	0.60	0.00	0.25	0.25	0.05	-0.17	-0.13	0.19
FR	-0.07	0.10	0.00	-0.01	0.00	0.00	0.03	0.13	-0.09	0.84	-0.41	1	-0.08	0.06	0.00	0.04	-0.07	0.17	0.05	0.00	0.16	-0.09	0.03	0.05	-0.06
FSV	0.18	0.20	-0.07	-0.13	-0.07	0.00	0.29	0.20	0.21	0.04	0.19	-0.09	1	-0.13	-0.29	0.81	0.94	0.02	-0.36	0.85	0.01	-0.36	-0.33	-0.31	-0.04
DFM	-0.05	-0.02	0.06	0.12	0.16	0.00	-0.15	-0.10	-0.11	0.08	0.02	0.06	-0.13	1	0.66	-0.06	-0.18	0.13	0.23	-0.11	0.02	0.12	0.16	0.23	-0.13
MP	0.02	-0.04	0.01	0.47	0.31	-0.01	-0.80	-0.40	-0.26	-0.05	-0.11	0.00	-0.29	0.69	1	-0.29	-0.35	-0.03	0.49	-0.29	-0.01	0.49	0.54	0.62	-0.20
TFW	0.20	0.27	-0.03	-0.13	-0.03	0.00	0.39	0.34	0.50	0.34	0.47	0.04	0.81	-0.06	-0.30	1	0.79	0.46	-0.29	0.84	0.23	-0.26	-0.32	-0.33	0.08
NFA	0.16	0.20	-0.11	-0.13	-0.06	0.00	0.32	0.25	0.19	0.04	0.18	-0.07	0.94	-0.18	-0.36	0.79	1	-0.06	-0.37	0.85	0.00	-0.35	-0.35	-0.35	0.01
AFW	0.09	0.15	0.09	-0.13	-0.02	0.01	0.27	0.27	0.64	0.57	0.61	0.17	0.02	0.13	-0.04	0.46	-0.06	1	0.02	0.13	0.42	-0.03	-0.13	-0.12	0.09
WHS	-0.03	-0.06	0.05	0.45	0.31	-0.01	-0.56	-0.36	-0.21	0.06	0.00	0.05	-0.36	0.23	0.50	-0.29	-0.37	0.02	1	-0.35	-0.11	0.71	0.77	0.75	-0.24
NSA	0.22	0.21	-0.08	-0.09	0.03	0.00	0.33	0.33	0.26	0.18	0.25	0.00	0.85	-0.11	-0.30	0.84	0.85	0.13	-0.35	1	0.43	-0.29	-0.38	-0.37	0.15
ASF	0.15	0.00	0.00	-0.03	0.09	0.00	0.22	0.29	0.26	0.37	0.25	0.16	0.01	0.02	-0.01	0.23	0.00	0.42	-0.11	0.43	1	-0.02	-0.26	-0.28	0.35
SL	0.01	-0.04	-0.02	0.62	0.41	-0.01	-0.63	-0.30	-0.27	-0.08	0.05	-0.09	-0.36	0.12	0.50	-0.26	-0.35	-0.03	0.71	-0.29	-0.02	1	0.79	0.62	0.10
SW	0.01	-0.05	-0.01	0.59	0.37	-0.01	-0.71	-0.45	-0.37	-0.05	-0.17	0.03	-0.33	0.16	0.56	-0.32	-0.35	-0.13	0.77	-0.38	-0.26	0.79	1	0.77	-0.44
ST	-0.02	-0.10	0.04	0.55	0.31	-0.02	-0.74	-0.48	-0.34	-0.01	-0.14	0.06	-0.31	0.23	0.64	-0.33	-0.35	-0.12	0.75	-0.37	-0.28	0.62	0.77	1	-0.32
SR	0.03	-0.02	0.04	-0.08	-0.07	0.01	0.23	0.31	0.15	0.00	0.19	-0.06	-0.04	-0.14	-0.20	0.08	0.01	0.09	-0.24	0.15	0.35	0.10	-0.44	-0.32	1

The characters that clustered closely or together were considered highly associated and those not featured were considered to have no contribution to the total variation (Fig. 1).

The first 4 factors were the most relevant as they provided the exact picture of total variability for each character (Mladenovic *et al.*, 2012b; Sinha and Kumaravadivel, 2016). The remaining factors were considered weak or had no discriminatory power because they explained only a quarter of the total variation (Maji and Shaibu, 2012). Odiyi *et al.* (2014) reported two factors accounting for more than 82% of the total variation. The high loads of the four factors and their corresponding characters indicated existence of high variation (Ebrahimnejad and

Rameeh, 2016), correlation and influence by similar gene(s) (Norman *et al.*, 2014). The inter-correlations or shared variance separated the characters into smaller groups that explained high variation in the original data set (Bhandari *et al.*, 2017). Hence, the characters were considered critical in contributing (Balkaya *et al.*, 2010a), and identifying genetic variability (Norman *et al.*, 2014).

The sign on the loads indicated the direction of the relationship between the characters (Balkaya *et al.*, 2010b). Odiyi *et al.* (2014) reported marketable leaf yield, vine length, branches per plant and leaves per plant as important characters of genetic variability in fluted pumpkins. In our study, the first four factors of greatest genetic variation were delineated by seed and

fruit characters. The functional relationships assigned to these factors were growth, yield and quality. Growth (Factor 1) was highly loaded for 100-seed weight, seed length, width and thickness. McCormack (2005) and Aruah *et al.* (2012) reported wide genetic variation and indistinguishable characteristics in seed characters. Balkaya *et al.* (2010a) and Mladenovic *et al.* (2012a) reported wide variation in seed length,

width and thickness and in 100-seed weight. Seed is the first link in the food chain, growth and the basic form of genetic variability. Seed size compensates for variation in environmental conditions in that as it increases there is more food reserve to sustain growth and variability. Food reserves of smaller seeds are quickly exhausted, thereby affecting seedling growth and variability.

Table 5. Direct (diagonal bold) and indirect genotypic path coefficients of different quantitative characters on total fruit weight of pumpkin accessions (residual effect = 22%)

	PS	IL	NFF	ST	LS	LR	DFP	PL	FT	FL	FW	FR	FSV	DFM	MP	NFA	AFW	WHS	NSA	ASF	SL	SW	ST	SR	TFWCC
PS	0.00	0.00	0.00	0.01	-0.01	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.03	0.01	0.00	0.01	0.07	0.00	0.16	-0.05	0.00	0.00	0.00	0.00	0.20
IL	0.00	0.00	0.00	0.00	-0.01	0.00	0.00	0.00	0.01	0.04	0.01	-0.01	0.03	0.00	0.00	0.01	0.06	0.00	0.15	0.00	0.00	0.00	0.02	0.00	0.27
NFF	0.00	0.00	0.02	0.00	-0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.01	-0.01	0.00	-0.01	0.04	0.00	-0.06	0.00	0.00	0.00	-0.01	0.00	-0.03
STT	0.00	0.00	0.00	0.05	-0.03	0.00	0.02	0.01	-0.02	0.00	0.00	0.00	-0.02	-0.01	-0.01	-0.01	-0.06	0.01	-0.06	0.01	0.07	0.00	-0.10	0.00	-0.13
LS	0.00	0.00	0.00	0.04	0.00	0.01	0.00	-0.01	0.01	0.00	0.00	0.00	-0.01	-0.02	-0.01	0.00	-0.01	0.01	0.02	-0.03	0.05	0.00	-0.06	0.00	-0.03
LR	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
DFP	0.00	0.00	0.00	-0.03	0.01	0.00	-0.02	-0.02	0.02	0.03	0.01	0.00	0.04	0.02	0.02	0.02	0.11	-0.01	0.24	-0.08	-0.07	0.00	0.13	0.01	0.39*
PL	0.00	0.00	0.00	-0.02	0.01	0.00	-0.01	-0.03	0.02	0.05	0.01	-0.01	0.03	0.01	0.01	0.01	0.11	-0.01	0.24	-0.11	-0.03	0.00	0.09	0.01	0.34*
FT	0.00	0.00	0.00	-0.01	0.01	0.00	-0.01	-0.01	0.06	0.06	0.04	0.01	0.03	0.01	0.01	0.01	0.27	0.00	0.19	-0.09	-0.03	0.00	0.06	0.00	0.50*
FL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.01	0.02	0.21	0.01	-0.05	0.01	-0.01	0.00	0.00	0.24	0.00	0.13	-0.13	-0.01	0.00	0.00	0.00	0.34*
FW	0.00	0.00	0.00	0.00	0.00	0.00	-0.01	-0.01	0.04	0.02	0.06	0.02	0.03	0.00	0.00	0.01	0.26	0.00	0.18	-0.09	0.01	0.00	0.02	0.00	0.47*
FR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.01	0.17	-0.02	-0.06	-0.01	-0.01	0.00	0.00	0.07	0.00	0.00	-0.06	-0.01	0.00	-0.01	0.00	0.04
FSV	0.00	0.00	0.00	-0.01	0.00	0.00	-0.01	-0.01	0.01	0.01	0.01	0.01	0.14	0.01	0.01	0.04	0.01	-0.01	0.61	0.00	-0.04	0.00	0.06	0.00	0.81*
DFM	0.00	0.00	0.00	0.01	-0.01	0.00	0.00	0.00	-0.01	0.02	0.00	0.00	-0.02	-0.11	-0.02	-0.01	0.06	0.00	-0.08	-0.01	0.01	0.00	-0.04	0.00	-0.06
MP	0.00	0.00	0.00	0.02	-0.01	0.00	0.02	0.01	-0.02	-0.01	-0.01	0.00	-0.04	-0.08	-0.03	-0.02	-0.02	0.01	-0.22	0.00	0.06	0.00	-0.11	0.00	-0.30*
NFA	0.00	0.00	0.00	-0.01	0.00	0.00	-0.01	-0.01	0.01	0.01	0.01	0.00	0.13	0.02	0.01	0.05	-0.03	-0.01	0.61	0.00	-0.04	0.00	0.06	0.00	0.79*
AFW	0.00	0.00	0.00	-0.01	0.00	0.00	-0.01	-0.01	0.04	0.12	0.03	-0.01	0.00	-0.01	0.00	0.00	0.42	0.00	0.09	-0.15	0.00	0.00	0.02	0.00	0.46*
WHS	0.00	0.00	0.00	0.02	-0.01	0.00	0.01	0.01	-0.01	0.01	0.00	0.00	-0.05	-0.03	-0.01	-0.02	0.01	0.02	-0.25	0.04	0.08	0.00	-0.13	-0.01	-0.29*
NSA	0.00	0.00	0.00	0.00	0.00	0.00	-0.01	-0.01	0.02	0.04	0.01	0.00	0.12	0.01	0.01	0.04	0.06	-0.01	0.72	-0.16	-0.03	0.00	0.07	0.00	0.84*
ASF	0.00	0.00	0.00	0.00	0.00	0.00	-0.01	-0.01	0.02	0.08	0.01	-0.01	0.00	0.00	0.00	0.00	0.18	0.00	0.31	-0.36	0.00	0.00	0.05	0.01	0.23
SL	0.00	0.00	0.00	0.03	-0.02	0.00	0.02	0.01	-0.02	-0.02	0.00	0.01	-0.05	-0.01	-0.01	-0.02	-0.01	0.01	-0.21	0.01	0.11	0.00	-0.11	0.00	-0.26
SW	0.00	0.00	0.00	0.03	-0.01	0.00	0.02	0.01	-0.02	-0.01	-0.01	0.00	-0.05	-0.02	-0.01	-0.02	-0.06	0.01	-0.27	0.09	0.09	0.00	-0.14	-0.01	-0.32*
ST	0.00	0.00	0.00	0.03	-0.01	0.00	0.02	0.01	-0.02	0.00	-0.01	0.00	-0.04	-0.03	-0.02	-0.02	-0.05	0.01	-0.27	0.10	0.07	0.00	-0.18	-0.01	-0.33*
SR	0.00	0.00	0.00	0.00	0.00	0.00	-0.01	-0.01	0.01	0.00	0.01	0.00	-0.01	0.02	0.01	0.00	0.04	0.00	0.11	-0.13	0.01	0.00	0.06	0.02	0.08

Yield (Factor 2), which was highly loaded for fruit size, total weight, number, and seeds per accession. Gichimu *et al.* (2008) and Balkaya *et al.* (2010b) reported variability in fruit characteristics. Variability has also been reported in fruits size and number per plant (Aruah *et al.*, 2012) and weight (Balkaya *et al.*, 2010b; Du *et al.*, 2011; Xiaohua *et al.*, 2011). Nerson (2007) observed large differences in seed number among and within fruit-types, Aruah *et al.* (2010) in seeds per fruit, and Gavilanez-Slone (2001) in seed number in proportion to pollen deposited on the stigma. McCormack (2005) and OECD (2012) reported that variation in seeds per fruit among species and varieties depends on pollination efficiency, area of the country and growing

conditions. Razim (2011) observed genotype variation depending on location, year and season; genotypes exhibited superior yield in one location, but were not stable in others with different agro-ecologies because of interaction between genotype and environmental factors. Quality (Factors 3 and 4) were highly loaded for fruit flesh thickness, width, length and length/width ratio. Balkaya *et al.* (2010b) reported variability in fruit diameter and length, while Mladenovic *et al.* (2012a) reported in fruit flesh thickness.

The high communality than specificity indicated that observed variation was influenced by many associated factors (Beaumont, 2012). The high (>50%)

communalities indicated high contribution of the characters to genetic variability. The ordination combined number of fruits and seeds, fruit total weight, size, flesh thickness, average weight, length, width, length/width ratio, seed length, width and thickness, and 100-seed weight into three groups of closely related characters (Norman *et al.*, 2014). This information facilitated detection and prediction of characters giving good description of the amount of genetic variability in the first three factors (Odiyi *et al.*, 2014).

Genetic variability

Genotypic and phenotypic variances: They varied from 0 for leaf length/width ratio to 385,376.4 for seeds per accession. The variances were greater than environmental variances (Table 3). Phenotypic variances exceeded genotypic variances in most characters, but equaled plant size, fruit size, total, average weight, number, 100-seed weight, seeds per accession or fruit, thickness, length/width ratio, and resulted in zero values in the environmental variances. The difference in range values was highest for days to first flowering (Table 3).

Genotypic variances were greater than environmental variances in all the characters, which indicated that the genetic component was the major contributor to total variation (Aruah *et al.*, 2012). Phenotypic variances were slightly greater than genotypic and environmental variances in most of the characters, suggesting little environmental influence on expression of these characters (Kiramana and Isutsa, 2016). Nwangburuka *et al.* (2014) reported phenotypic variance greater than genotypic and environmental variances in all the characters. Kiramana *et al.* (2016) reported genotypic and phenotypic variances greater than environmental variances in most characters. In our study, genotypic and phenotypic variances that were equal and led to zero environmental variances were observed in some characters, implying that offspring produced would be exactly like their parents, because of no visible environmental effects on their expression (Ogunniyan and Olakojo, 2014). Jonah *et al.* (2013) reported

genotypic and phenotypic variances of equal magnitude in Bambara nut pod width, length and seed width, while Ogunniyan and Olakojo (2014) reported genotypic and phenotypic variances equal in days to anthesis and silking in maize. The low and moderate genotypic, phenotypic and environmental variances observed in certain characters indicated their stable nature (Fukrei *et al.*, 2011), and little environmental influence on their expression (Roychowdhury and Tah, 2011). Nwangburuka *et al.* (2014) reported low genotypic, phenotypic and environmental variances for leaf length and width, petiole length, number of branches and leaflets, fruit yield, seed length, width and weight, but moderate values for fruit number, length and width in fluted pumpkins.

The continued selection and use by farmers could have resulted in the traditional pumpkin remaining stable (Fukrei *et al.*, 2011). The high genotypic and phenotypic variances suggested adequate gain in selection on the characters, meaning heterosis could be utilized to produce superior hybrids and genotypes (Kiramana and Isutsa, 2016). Selection based on such characters can achieve broad genetic variation and improvement of pumpkins (Akter *et al.*, 2013). Aruah *et al.* (2012) reported high genotypic and phenotypic variances in fruit weight, diameter and seeds, while Sultana *et al.* (2015) in fruit length, first male and female flower timing. Genotypic, phenotypic and environmental variances for leaf length/width ratio were zero, thus selection based on this character would not be effective for any intended improvement (Kiramana and Isutsa, 2016). Nwangburuka *et al.* (2014) reported zero genotypic, phenotypic and environmental variances for vine width in fluted pumpkin, while Ene *et al.* (2016) reported for mean fruit weight in cucumber. The high difference for genotypic and phenotypic variances versus environmental variances indicated that variability was not only genetic, but also environmentally influenced. Selection based on such characters would not be effective for improvement of pumpkins due to masking of genotype expression by environment and non-additive gene effects (Kiramana and Isutsa,

2016). High environmental variances in germination percentage, flowering and fruit maturity timing have also been reported (Kiramana and Isutsa, 2016). The high environmental, genotypic and phenotypic variance differences were attributed to the interaction between the genotypes and environment (Mohsin *et al.*, 2017). OECD (2012) reported that flower development is regulated by genetic and environmental factors such as temperature and day duration.

Genotypic and phenotypic coefficients of variation: They ranged from 5.4% for days to first fruiting to 83.5% for fruit size under GCV, and 88% for leaf length/width ratio under PCV (Table 3). PCV was higher than GCV in most traits, but equaled each other in plant size, fruit size, total, average, number, 100-seed weight, seed number, average, thickness and length/width ratio. The GCV and PCV were highest for fruit size, number per accession, total weight and number of seeds; the least for days to first fruiting, and leaf length/width ratio under GCV, seed length/width ratio under both GCV and PCV in that order. The differences in range between PCV and GCV were above 1 for internode length, nodes to first fruit, days to first flowering, fruits and leaf length/width ratio, and below 1 in all other traits (Table 3).

The slightly higher PCV than corresponding GCV for most characters reflected more contribution by the genotype than environment in expression of the characters (Nagare *et al.*, 2017), and selection based on phenotypic characters was feasible (Ene *et al.*, 2016). The slight difference between PCV and GCV was attributed to planting of the accessions under similar environmental conditions in Chuka University research farm. Nwangburuka *et al.* (2014) reported slightly higher PCV than GCV in all characters, while Nagar *et al.* (2017) reported in most traits of pumpkins. PCV and GCV values greater than 20% are regarded as high, between 10% and 20% as medium, and less than 10% as low (Khan *et al.*, 2016). Based on this fact, most of the characters in our study were categorized as high, followed by moderate, and lastly low. The variation of the characters from low,

moderate to high PCV and GCV indicated presence of genetic variability (Nagar *et al.*, 2017). The high PCV in characters indicated the existence of wide scope of selection for the improvement of the traits (Yadav, 2000), while the low PCV presented less scope for selection (Khan *et al.*, 2009).

Nagare *et al.* (2017) reported high PCV and GCV values for plant weight, mature fruit weight and yield, moderate PCV and GCV values for peduncle length, vine length, and low PCV and GCV values for days to marketable maturity, days to first female or male flower opening, node number of first female or male flower. Rahman *et al.* (2002) reported high PCV and GCV values for fruit yield, number, length, flesh thickness, and stem length, while Saha *et al.* (1992) reported high GCV in fruit weight, length and yield in pumpkins. Ene *et al.* (2016) observed highest PCV and GCV for number of branches, vine length, fruit weight and leaf area. The large PCV and GCV values revealed their use in selection of suitable parents for crossing, or lines for improvement of pumpkin yields (Ogunniyan and Olakojo, 2014).

The low PCV and GCV values indicated there was least chance of modifying pumpkin accessions using the characters (Mohsin *et al.*, 2017). Ene *et al.* (2016) observed low PCV and GCV for days to 50% female flowering, while Nagar *et al.* (2017) for days to marketable maturity stage, days to first female or male flower opening, and node number of first female or male flower. The PCV and GCV values were that were equal, indicated that there was interaction between the accessions with the environment (Jonah *et al.*, 2013). Ogunniyan and Olakojo (2014) reported equal PCV and GCV values for days to anthesis and silking in maize, while Jonah *et al.* (2013) for pod width, length and seed width of Bambara nut.

The range between PCV and GCV for all characters was narrow, except for leaf length/width ratio, implying that genotype contributed more than environment in expression of these characters and selection based on phenotypic values is therefore feasible (Aruah *et al.*, 2012). Nagare *et al.* (2017)

reported small ranges between PCV and GCV in majority of the characters in pumpkin, while Nwangburuka *et al.* (2014) in all characters of fluted pumpkin. The wide range for leaf length/width ratio indicated a greater degree of environmental control (Ene *et al.*, 2016). High environmental influence on a character reduces its response to selection on phenotypic basis (Islam *et al.*, 2009).

Heritability and genetic advance (gain): Heritability ranged from 12% for leaf length/width ratio to 100% for plant size, fruit size, total, average weight, number, 100-seed weight, seed number per accession, average per fruit or accession, thickness and length/width ratio (Table 3). Heritability exceeded 80% in all the characters, except leaf length/width ratio and days to first flower. Genetic advance was highest for seeds per accession and lowest for seeds and fruit length/width ratio. Expected genetic advance was highest and lowest for fruit size and leaf length/width ratio, respectively. High heritability corresponded to high genetic advance for fruit size, total weight, number and seeds per accession, while low heritability corresponded to low genetic advance for leaf length/width ratio (Table 3).

Heritability estimates give an insight into the extent of genetic control to express a particular trait and phenotypic reliability in predicting its breeding value (Ene *et al.*, 2016). In our study, the 100% heritability indicated that the observed variation was highly contributed by the genotypic component (Kumar *et al.*, 2014), with less influence by the environment (Ogunniyan and Olakojo, 2014). Hence, phenotype could provide a perfect measure of the genotypic value and the characters would respond to selection (Jonah *et al.*, 2013). The characters can also be given special attention during selection aimed at improving pumpkins (Ene *et al.*, 2016). Kiramana *et al.* (2016) reported heritability estimates ranging from 71.9% for number of male flowers to 95.3% for 100-seed weight, and from 21.3% for germination percentage to 86% for total fruit weight. According to Khan *et al.* (2016), heritability values greater than 80% are very high, 60% to 79% are moderately high, 40% to 59% are

medium and less than 40% are low. The high heritability observed indicated less environmental influence and considerable genetic variation that warrant selection for improving pumpkin.

Kiramana *et al.* (2016) reported highest expected genetic advance for total fruit weight and lowest for fruit width and germination percentage. According to Khan *et al.* (2016) expected genetic advance values 0% to 10% are low, 10% to 20% are moderate, while 20% and above are high. The high heritability coupled with high expected genetic advance indicated predominance of additive gene effect. Thus, joint consideration of heritability and genetic advance during selection would be effective to improve pumpkins (Khan *et al.*, 2016). The low heritability and expected genetic advance indicated predominance of environmental effect. Therefore, selection of such a character would be ineffective to improve pumpkins. The high heritability and moderate expected genetic advance indicated non-additive gene action (Malashetty, 2010), making it difficult to select such a character to improve pumpkins due to environment masking expression of genotypic effect (Aruah *et al.*, 2012). Kiramana and Isutsa (2016) reported high heritability and expected genetic advance for all characters, except number of leaves and fruit length.

Character association

Genotypic and phenotypic correlations: The analysis identified both positive and negative genotypic and phenotypic correlations, with bolded ones being significant at $P=0.05$ (Table 4). The genotypic correlations were higher than the corresponding phenotypic ones in most characters. Numbers of nodes to the first flower and leaf length/width ratio correlations with all other characters were not significant genotypically and phenotypically. Significant correlations were observed in some characters phenotypically, but not genotypically. Similar correlations were observed in fruit size, total, average weight, number, 100-seed weight, seed number, average, length, width and thickness at both levels. Details of how factors were associated

genotypically and phenotypically are shown in Table 4.

In our study, genotypic correlation coefficients greater than phenotypic ones indicated greater contribution of genetic factors to development and inherent association of characters (Kassahun *et al.*, 2013; Fayeun and Odiyi, 2016). The high genotypic correlations were attributed to overlap of environmental factors by genetic ones (Avijala *et al.*, 2015), acting in the same direction to maximize phenotypic expression (Chaudhari *et al.*, 2017). The low phenotypic correlation coefficients were as a result of environmental effects modifying the association of characters at genotypic level (Nzuve *et al.*, 2014). Genotypic correlations devoid of environmental correlations are more useful than phenotypic correlations in deciding selection strategies (Fayeun and Odiyi, 2016). Genotypic correlations involve association of heritable characters as they determine higher interest of genetic breeding (Machado *et al.*, 2017), while phenotypic correlations are made up of genotypic and environmental correlations (Fayeun and Odiyi, 2016). Characters that exhibit greater genotypic than phenotypic correlations are favourable for selection (Avijala *et al.*, 2015). Higher genotypic than phenotypic correlations were reported by Kassahun *et al.* (2013) in coriander, Avijala *et al.* (2015) in cassava, and Fayeun and Odiyi (2016) in fluted pumpkin.

Since most of the characters were significant at both levels they indicated less environmental influence and hence phenotypic correlations were good indicators of genotypic correlations (Fayeun and Odiyi, 2016). Non-significant correlations at both levels indicated minimal and non-significant contribution to development and yield of pumpkins. Sultana *et al.* (2015) reported non-significant correlations for pedicel length of female flower with most of the agronomic traits evaluated. Significant phenotypic correlations relative to non-significant genotypic counterparts for certain characters suggested little environmental influence on the expression of the

characters (Fayeun and Odiyi, 2016). The same correlation trends at both levels were observed in some characters in our study probably due to planting of all the accessions in one farm under uniform conditions that could have decreased environmental variation and inflated heritable and genetic correlations. Waitt and Levin (1998) reported similar genotypic and phenotypic correlations in plants, while Chaudhari *et al.* (2017) reported the same correlation trends at both levels in most pumpkin traits.

The observed significant and positive correlations at both levels suggested simultaneous improvement of these characters because of mutual relationships, and selection for one would translate to selection and improvement of the other (Fayeun *et al.*, 2012). The sign of correlation indicated how characters impacted each other, with positive sign indicating acceleration, while negative sign indicating de-acceleration (Kiramana and Isutsa, 2017). Nzuve *et al.* (2014) inferred that when correlations are positive, genes controlling the characters are linked or positioned closely together on the same chromosome or could be under the control of pleiotropic genes. Positive relationships provide good selection indices (Fayeun and Odiyi, 2016) for desirable genes that influence inheritance of characters that could be exploited for further improvement (Nzuve *et al.*, 2014). An increase in characters showing positive and significant correlations could lead to enhanced pumpkin yields (Nzuve *et al.*, 2014). These characters could be utilized as yield indicators when performing selection (Sampath and Krishnamoorthy, 2017). Our study results implied that selection for fruit flesh thickness, length, diameter and weight would result in improvement of fruit yield (Mahaveer *et al.*, 2017) and average weight per plant (Grisales *et al.*, 2015). Mahaveer *et al.* (2017) and Shivananda *et al.* (2013) reported positive associations at both levels of fruit yield with number, circumference, average weight, length, cavity size, flesh thickness, vine length, seed number, and 100-seed weight. Total fruit weight exhibited significant, but low and negative correlations with maturation period, 100-seed weight, seed width and thickness at both levels, implying that

pumpkin yields cannot be improved based on selection of these characters. The negative correlations implied that an increase in one variable would lead to a decrease in the other (Bolina *et al.*, 2013). Mahaveer *et al.* (2017) reported negative and significant associations of fruit yield with days to first male and female flowers. In our study, genotypic and phenotypic correlations that were either zero or insignificant suggested that the characters were independent (El-Mohsen *et al.*, 2012).

Path coefficient analysis: Total fruit weight was taken as a dependent variable and the rest of the characters as independent variables. The number of seeds per accession showed the highest positive and direct effect on total fruit weight, followed by fruit average weight, length, size and seed length. The highest negative and direct effect on total fruit weight was exhibited by average seeds per fruit, followed by seed thickness and days to fruit maturity. All other associations of characters revealed by path coefficient analysis with total fruit weight are shown in Table 5. The sum of direct and indirect effects for each character was equal, and/or plus or minus 1 the total fruit weight genotypic correlation coefficients. The sum was highest and positive for number of seeds, followed by fruit size and number, but lowest and positive for leaf length/width ratio. The sum was negative for number of nodes to the first flower, stem thickness, leaf size, and days to first mature fruit, maturation period, 100-seed weight, seed length, width and thickness, with all other characters exhibiting positive direct and indirect effects. The residual effect factor was 22% (Table 5).

In our study, the highest positive and direct effect indicated major contribution to total fruit weight (Naik *et al.*, 2015). Aruah *et al.* (2012) reported highly positive and direct effect of number of female flowers on fruit weight. Naik *et al.* (2015) reported maximum direct effect of fruit length and weight, seed cavity and vine length on yield of pumpkin at genotypic level, while Tiwari and Upadhyay (2011) reported highly positive and direct effect of fruit weight, number, average weight, and number of primary branches with

fruit yield. Despite highly positive and direct effects, seed length failed to obtain a significant correlation with total fruit weight, because it was masked by strong negative and indirect effects of number of seeds, seed thickness and other characters. The negative correlation of seed length with total fruit weight suggested simultaneous restricted selection should be imposed to nullify the undesirable indirect effects to make use of the high direct effect of seed length. Number of nodes to the first flower, stem thickness, 100-seed weight and seed width failed to exert significant correlation with total fruit weight probably due to weak direct and indirect positive effects that were masked by strong and simultaneous negative indirect effects of other characters (Malashetty, 2010). The direct effect showing a narrow range suggested true relationship. Therefore, direct selection for hybridization based on these characters could result in appreciable improvement of total fruit weight (Tiwari and Upadhyay, 2011; Naik *et al.*, 2015). The low and positive direct effect, as well as high and positive correlation of fruit number and size with total fruit weight reflected fake association of these characters. The high indirect effect through seed number indicated that total fruit weight could be improved by indirect selection of seed number (Shivanada *et al.*, 2013).

Days to first flowering and peduncle length had negative and paltry direct effect, as well as positive correlation with total fruit weight, suggesting indirect effects were the causes of this correlation. Therefore, indirect causal factors of these characters should also be considered during selection (Malashetty, 2010). Plant size and seed length/width ratio showed low and positive direct effects and non-significant correlations with total fruit weight. The positive and indirect effects of these characters through average fruit weight and number of seeds, as well as simultaneous positive and direct effects through other characters nullified the negative and indirect effects, but failed to establish significant associations with total fruit weight (Shivanada *et al.*, 2013). Likewise, strong negative and direct effect of seed thickness and days to fruit maturity, coupled with negative and

indirect effect via other characters, failed to nullify the positive and indirect effects to total fruit weight (Malashetty, 2010). Therefore, selection based on plant size, seed length/width ratio, seed thickness and days to fruit maturity is not recommended for enhancing total fruit weight (Grisales *et al.*, 2015).

Average seed size had strong and negative direct effect on total fruit weight. However, the high and positive indirect effect of average seeds via average fruit weight and number of seeds, overcame the negative and indirect effect of days to first flowering, peduncle length and fruit length/width ratio to establish a positive and significant association with total fruit weight. Similarly, days to first flower, peduncle length and fruit length/width ratio had weak and negative direct effects, as well as high and positive indirect effects via other characters. The high and positive indirect effects of these characters overcame the negative and indirect effects to establish positive associations with total fruit weight (Malashetty, 2010). Leaf size and maturation period had weak and negative direct effects and correlations with total fruit weight. The positive and indirect effects of these characters failed to nullify the negative and direct and indirect effects to establish significant positive associations with total fruit weight. Therefore, indirect selection of these characters would not help in reducing undesirable effects on total fruit weight (Shivanada *et al.*, 2013).

Internode length, fruit width and flesh thickness had weak and direct positive effects on total fruit weight. The significantly positive correlation of these characters with total fruit weight was mainly due to indirect positive effects via average fruit weight and number of seeds per accession. The negatively indirect effects of these characters via other characters failed to nullify the positively direct and indirect effects. Hence, these characters were able to establish positive and significant associations with total fruit weight. Sampath and Krishnamoorthy (2017) reported negative and direct effects of number of primary branches, petiole length, leaf breadth, first male flower node, fruit diameter, flesh thickness and

100-seed weight on pumpkin yield. Sultana *et al.* (2015) observed highly negative and direct effects of leaf breadth and pedicel length of female flower on pumpkin fruit yield. Malashetty (2010) observed weak and negative direct effect of number of primary branches and indirect effects via days to first harvest, fruit number and node of first female flower on fruit yield. Leaf length/width ratio failed to establish a significant correlation with total fruit weight because of weak directly positive and insignificant indirectly positive effects via all other characters.

Path coefficient analysis revealed that the seeds, fruit average weight, length, size, number, flesh thickness, peduncle length, days to first flower should be considered simultaneously in total fruit weight improvement (Tiwari and Upadhyay, 2011; Kumar *et al.*, 2017). The residual effect determines how best causal factors account for variability of the dependent factors (Ahmed *et al.*, 2018). In our study, about 78% of the total fruit weight was contributed by characters studied (Khan *et al.*, 2016). Other factors contributing 22% effect were not included in this study (Sultana *et al.*, 2015). Therefore, more factors need to be considered when selecting pumpkins for high total fruit weight (Tiwari and Upadhyay, 2011). Sultana *et al.* (2015) and Khan *et al.* (2016) reported 38% and 9.25% residual effects, respectively.

Conclusions and Recommendations

Morphogenetic variability was effectively assessed using multivariate analysis techniques. Analysis of variance, mean and range showed significant variations in all the characters studied. Range values more than double the mean indicated existence of adequate morphological variability. The economically interesting characters with high mean values should be selected for improvement of pumpkins. Factor analysis produced 8 factors, with fewer latent characters sharing a common variance. The characters with high scores on the first four factors could be used as good genitors and priority indices in screening and selecting pumpkins for improvement. Three dimensional ordination classified characters into clusters. The characters clustered together could

have heterosis useful for breeding hybrids in pumpkins. Those characters far from the rest are genetically variable due to crossing of different pumpkins and important for identifying accessions. The PCV and GCV should be investigated concomitantly with heritability and genetic advance, because they alone are not able to determine genetic variability for credible selection of best performing pumpkins. Thus to improve pumpkins, direct selection can be made on fruit size, total weight, number and seeds that showed highly positive correlations at both levels. Path coefficient analysis of seeds, fruit average weight, length, and size showing highly direct effects, and fruit number, flesh thickness, length, peduncle length and days to first flower showing highly indirect effects should be considered simultaneously for pumpkin total fruit weight improvement. Multivariate analysis techniques revealed fruit size, number, total weight and seeds as characters of genetic variability, with high potential for successful and efficient selection for better yields. Since these findings are based on accessions collected in two regions only, collection and assessment of variability in landraces from other regions is recommended.

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