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Research Article

Diversity in bottle gourd (*Lagenaria siceraria* - (Molina) Standl.) Germplasm from peninsular india

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Abstract
A set of 20 diverse accessions of bottle gourd (*Lagenaria siceraria* (Molina) Standl.), exhibited wide range of variability for qualitative and quantitative traits. The seed oil content ranged from 18.6 % (IC446598) to 28.0 % (IC446592). The fatty acid composition of bottle gourd seed oil also varied. Days to 50% flowering and peduncle length had significant positive correlation with seed oil content, whereas inter nodal length had significant negative correlation. The intermodal length and number of primary branches per plant had high positive direct effect on fruit yield per plant. Based on the traits, the accessions clustered into three distinct clusters. The diversity may be exploited for crop improvement and the potential of bottle gourd as edible oil source further explored.

Key words: Bottle Guard, correlation, path analysis, diversity, seed oil

Introduction

Bottle gourd, synonymous to white flowered gourd and calabash gourd (*Lagenaria sicereria* (Molina) Standl.), is one of the important cucurbits grown throughout the world for its tender fruits (Arvind *et al.*, 2011). It is grown in rainy season as well as in summer season. *L. siceraria,* generally cultivated in all tropical parts of the world including India and few African countries. *L. siceraria* is reported to have 2n = 2X = 22 chromosomes (Mayura *et al*., 2009). It is an annual, vigorous climbing species, monoecious and highly cross-pollinated having wide genetic variability across the globe. Yetisir *et al.* (2008) vividly summarised the collection and characterization bottle gourd germplasm (162 accessions) collected in 15 surveys from southern Turkey. Mladenovic *et al.* (2012) studied the landraces of bottle gourd from Balkan Peninsula and compared them to those from Africa, Asia and America. They observed that reduction in trait variation in bottle gourds was due to the preference for certain shapes and sizes of the fruit.

India has a rich genetic variability in terms of fruit size and shape (Sivaraj and Pandravada, 2005; Peter *et al.*, 2007) and is one of the centres of diversity according to de Candolle (as quoted in Chadha and Lal, 1993). However, very little work was done to document the variability for various traits in germplasm in India. Mathew *et al.* (2000) studied 28 accessions from different parts of India for the variability and concluded that there exists a potential source of gene sanctuary for bottle gourd that can be harnessed. Resistant sources for zucchini yellow mosaic virus and tolerance to powdery mildew from Indian collections were reported by Levi et al. (2009). Also, Padiyar and Singh (2009) studied the genetic diversity in 14 genotypes and reported that seed protein electrophoresis can be used for identifying genotypes in bottle gourd.

Collection of genetic resources and systematic study of the nature and magnitude of variability present in the germplasm and association among the various characters existing forms the basis for any crop improvement program. Hence, the present investigation was undertaken to collect and study the variability among the bottle gourd germplasm from the tribal areas of Peninsular, India.

Material and Methods

A gourd specific exploration survey covering different eco-geographical regions of Peninsular India was undertaken during 2007 and 2008. Twenty germplasm lines were collected by random sampling method. The sites of collection of the 20-germplasm lines are presented in Figure 1. The germplasm collected were evaluated along with four local controls *viz*., Arka Bahar (Indian Institute of Horticulture Research), Pusa Naveen

(Indian Agricultural Research Institute), Biogaurav and Swati (hybrids released by private companies- Bioseed and Sungrow seeds respectively) in an augmented block design with five blocks during summer 2009 at Vegetable Research Station, Agriculture Research Institute, Rajendranagar, Hyderabad. In each block, particular genotype was grown in a single row plot of 2.5 m length and 3 m width. Plants were grown with 3.0 (between-row) x 0.5 (in-row) m spacing, accommodating five plants per genotype. Observation for 25 qualitative traits and quantitative traits was carried out as per the minimal descriptors of bottle gourd (Srivastava *et al.*, 2001). In each genotype, observations were recorded on three randomly selected plants for all the traits except days to 50 % flowering, total number of fruits per plant, number of marketable fruit per plant, fruit yield per plant which were recorded on whole plot basis.

Estimation of oil content and composition of seed oil:Seed was extracted from mature fruits of three randomly selected plants, shade dried and was evaluated for oil content and composition. A well mixed seed (5.0 g) was ground and transferred into an extraction thimble and the top portion was covered with cotton. This packed thimble was placed in the extraction chamber of SER 148 Solvent Extractor (VELP Scientifica, Italy). Around 70 ml of hexane was taken in the extractor and the temperature of the solvent heating block was adjusted to 130ºC (Recommended set point for hexane). The thimble was socked in hexane and the solvent was refluxed over a period of one hour. After one hour, the thimble was lifted from the solvent and the solvent was allowed to pass through the bed of ground seeds for 15 min. This operation ensures washing of the thimble with fresh solvent. Hexane was distilled off to recover seed oil. A recovery of 1.40 g, corresponds to 28%. The fatty acid composition of bottle gourd was determined using a gas chromatograph (Agilent 6890) equipped with a flame ionization detector (FID) on a split injector system (Ahmed *et al.*, 2009). Fused silica capillary column (DB-225, 0.25µm, 30 m x 0.32 mm id) was used for the analysis. Oven temperature was programmed at 160ºC for 2 min, increased to 180ºC at 6ºC/min, held for 2 min and finally increased to 230ºC at 4ºC/min and held for 15 min. The injector and detector temperatures were held at 220 and 250ºC, respectively. Nitrogen was used as carrier gas at a flow rate of 1 ml/min. The area percentages were recorded with Agilent chemstation data processing system (Sunil, 2010).

The mean data on various biometric traits were subjected to analysis of variance of augmented block design as per the standard statistical procedure suggested by Federer (1956). Simple correlation coefficients were worked out among 17 growth, earliness and yield attributes using mean values to identify the yield components in bottle gourd. Correlation analysis was carried out according to the procedure given by Panse and Sukhatme (1985). In addition the direct and indirect effects of various independent quantitative traits on dependent fruit yield were also worked out by path analysis according to the procedure given by Panse and Sukhatme (1985). Multivariate cluster analysis of the accessions by ward's minimum method was carried out using the SAS Enterprise guide.

Results and Discussion

The collected accessions exhibited a wide range of morphological diversity for qualitative as well as for quantitative traits. In the present study, of all the traits studied, fruit and associated traits emerged as the most apparent and distinguishing morphological traits. This supplements the earlier findings of Sakar (2004) and Yetisir *et al.* (2008) who stated that this is the important identification trait for the species. Large variation was found in such a small set of 20 accessions. This may be attributed to distinct and diverse genetic base among Asian landraces as reported by Decker-Walters *et al.* (2001) and Decker-Walters *et al.*(2004).

Correlation among the characters Correlation coefficient analysis (Table 1) revealed that characters such as fruit length, fruit width, fruit weight, total number of fruit per plant, number of marketable fruits per plant had strong correlation with marketable yield per plant, suggesting that fruit yield could be improved by making selection on the basis of the afore said characters, which are considered as major yield attributes in bottle gourd as reported by Raja *et al.* (2006). Significant negative correlation was observed for internodal length with peduncle length (-0.44) and seed oil content (-0.40). Days to 50% flowering had significant negative correlation with peduncle length (-0.78), total number of fruits per plant (-0.44) and seed oil content (-0.47). Fruit length recorded significant negative correlation with fruit width (-0.68). Also, fruit weight was found to be negatively correlated with the total number of fruits per plant (-0.79). Significant positive correlation was observed for total number of fruits and number of marketable fruits per plant (0.69). The trait 100-seed weight was significantly correlated with fruit yield per plant (0.65). Pradeep and Syamal (2010) reported similar findings in their character association studied in bottle gourd.

Peduncle length had significant positive correlation ($p>0.05$) with seed oil content suggesting that greater the peduncle length more will be the translocation of photosynthetic assimilates produced in the peduncle which are directed for seed development. Inter nodal length had significant negative correlation with seed oil content, whereas the internodal length and number of primary branches per plant had high positive direct effect on fruit yield. Taha *et al.* (2003) have also reported that higher inter nodal length facilitates new fruit development. The negative correlation of internonal length with seed oil and positive correlation with fruit yield supports the view of Adams (1967) that contribution of components of yield is through component compensation mechanism i.e., seed oil content and fruit yield in this case. The high negative correlation of the days to 50% flowering to seed oil content could mostly be explained by the duration required for fruit and seed development.

Path analysis Among the 16 independent quantitative traits studied for their direct effects on fruit yield per plant (Table 2), the traits such as vine length, seed length to width ratio had high and negative direct effect on yield per plant, while internodal length and number of primary branches per plant had high positive direct effect. The traits such as petiole length had positively moderate direct effects. In contrast, fruit length and number of marketable fruits per plant had negatively moderate direct effect on yield per plant. The characters days to 50 % flowering, first female flowering node, days to first fruit harvest, fruit width, number of seeds per fruit and 100 seed weight had low positive direct effect on fruit yield per plant. The characters petiole length, fruit weight and total number of fruits per plant had negligible direct effects on yield per plant. The result of the present study also showed that internodal length and number of primary branches have direct effect on yield, was in slight variation to earlier findings of Yogesh *et al*. (2010) who reported that fruit weight, number of fruits, number of nodes per vine, length of fruit and days to first female flower had high direct effect on yield in bottle gourd genotypes. The present finding also highlights the high negative direct effect of vine length and seed length to breadth ratio as the latter confirms the earlier findings (Yogesh *et al.,* 2010).

Seed oil content and fatty acid profile The data on the bottle gourd seed oil content and composition is presented in Table 3. Significant variation was recorded for oil content as it ranged from 18.64 % (IC446598) to 28.02 % (IC446592) whereas, oil content of standard controls ranged from 20.93 % (Arka Bahar) to 27.83 % (Swati). The fatty acid composition of bottle gourd seed oil was also found to be variable. The highest values for saturated fatty acids, such as palmitic acid, were recorded in IC546149 (17.9 %) and lowest in IC546146 (12.4). The values for oleic acid ranged from 5.6 % to 10.6 %. The highest value for polyunsaturated fatty acid, linoleic acid was recorded in IC546175 (73.3 %) and lowest was recorded in IC546150 (63.3%), respectively. According to FAO, certain taxa within Curcubitacae, and in particular those that grow wild in arid areas, could be a potential source of oil and bottle gourd has also been found to be a potential minor oilseed crop. Paris and Nerson (2003) in their study with *Cucurbita pepo* concluded that globose fruits had the longest and flattest seed whereas cylindrical types had shortest and thickest seed which may serve as an indicator for the seed oil types. Gong *et al.* (2012) in their study on 104 accessions of *Cucurbita pepo* have identified specialised accessions including those suitable for seed oil extraction. These seed oil accessions were said to be derived from recessive hull-less mutation (Teppner, 2000).The quality of oil depends upon the function and composition of its fatty acid components. Earlier reports (Axtell, 1992) have shown that the seed oil of bottle gourd is similar to that of sunflower oil. Bottle gourd seeds were reported to be good source of lipids and proteins (Loukou, 2007). The fatty acid profile *viz.*, palmitic, stearic, oleic, linoleic, linolenic and arachidic varied among the germplasm under study, but were in line with the earlier findings as it had more unsaturated linoleic acid content than saturated fats.

Cluster Analysis The cluster analysis using the ward's minimum method resulted in three broad clusters (Figure 3). Cluster I comprised of 6 accessions: IC446593, IC446592, IC546181 and IC546170, plus two local controls, Swati and Pusa Naveen. Cluster II had 8 accessions: IC546150, IC546178, IC446599, IC446596, IC546168, IC546175, IC546160 and IC546149.Cluster III again comprised of two local controls, Arka Bahar and Bio gaurav, and 8 other accessions: IC570400, IC570480, IC546182, IC570504, IC446598, IC446600 and IC546146. The clustering of the accessions was not correlated with the geographical locations as for example IC446593 and IC546146, the two most diverse accessions in the present study were collected from Nizamabad and Adilabad district of Andhra Pradesh respectively, which form contiguous region. This also underscores the point that individual preferences and choices play significant role in the cultivation of bottle gourd. Cluster analysis also identified six accessions in cluster I and eight accessions in cluster III which grouped together the local controls and which can be used in transferring of biotic or abiotic traits from these accessions to the respective local controls. Cluster III emerged as distinct cluster which can be used in the crosses with other cluster for tapping hybrid vigour.

The correlation and path analysis indicated that vine length, seed length and width ratio, intermodal length and number of primary branches per plant are important selection indices for fruit yield per plant. The studied germplasm has high variation for all traits studied including seed oil content and fatty acid profile. Apart from its use as a vegetable and a medicinal plant, the other potential use of the crop as an edible minor oilseed, having up to 28% seed oil content and composition on par with sunflower oil, may be tapped.

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Table 1. Association among growth, earliness and yield parameters of bottle gourd.

Character	Internodal length (cm)	No. of primary branches	Petiole length (cm)	Days to 50 % flowering	First female flowering node	Days to first fruit harvest	Peduncle length (cm)	Fruit length (cm)	Fruit width (cm)	Fruit weight (g)	Total number of fruits per plant	No. of marketable fruits per plant	Seed length to breadth ratio	No. of seeds per fruit	100 seed weight (g)	Fruit yield per plant (kg)	Seed oil content (%)
Vine length (cm)	0.22	-0.08	-0.05	0.01	-0.12	0.14	-0.1	-0.22	0.07	-0.2	0.22	0.3	-0.34	-0.15	0.23	0.22	-0.3
Internodal length (cm)		-0.2	0.12	0.27	-0.35	0.03	-0.44*	0.3	-0.24	0.15	-0.07	0.1	0.12	0.06	0.11	0.11	-0.40*
No. of primary branches			0.06	0.12	-0.23	0.22	-0.21	0.28	-0.23	0.19	-0.03	-0.05	-0.01	0.09	0	-0.09	-0.03
Petiole length (cm)				0.47*	-0.41*	0.28	-0.50*	0.15	-0.41*	0.37	-0.38	-0.02	0.04	0.1	-0.25	-0.27	0.2
Days to 50 % flowering					-0.72*	0.50*	-0.78*	0.48*	-0.46*	0.47*	-0.44*	-0.24	0.17	0.36	-0.14	-0.32	-0.47*
First female flowering node						-0.21	0.554*	-0.33	0.249	-0.211	0.113	0.082	-0.037	-0.239	-0.015	0.174	-0.21
Days to first fruit harvest							-0.61*	0.53*	-0.66*	0.62*	-0.56*	-0.25	0.29	0.37	-0.22	-0.35	0.05
Peduncle length (cm)								-0.51*	0.56*	-0.48*	0.41*	0.17	-0.18	-0.32	0.14	0.23	0.44*
Fruit length (cm)									-0.68*	0.51*	-0.49*	-0.46*	0.17	0.56*	-0.54*	-0.48*	0.37
Fruit width (cm)										-0.66*	0.59*	0.37	-0.17	-0.36	0.23	0.48*	0.18
Fruit weight (g)											-0.79*	-0.47*	0.3	0.64*	-0.47*	-0.69*	0.28
Total number of fruits per plant												0.69*	-0.09	-0.51*	0.55*	0.64*	-0.04
No. of marketable fruits per plant													0.1	-0.47*	0.54*	0.52*	0.29
Seed length to breadth ratio														0.01	-0.01	-0.3	0.18
No. of seeds per fruit															-0.58*	-0.43*	-0.21
100 seed weight (g)																0.65*	0.12
Fruit yield per plant (kg)																	0.14

* Significant at 5% level

Table 2. Direct effects of various quantitative traits on fruit yield of bottle gourd.

Character	Path coefficient (direct effect)	Correlation with yield
Vine length (cm)	-1.190 H	0.223
Internodal length (cm)	1.358 H	0.116
No. of primary branches	0.735 H	-0.095
Petiole length (cm)	0.283 M	-0.274
Days to 50 % flowering	0.100 L	-0.323
First female flowering node	0.170 L	0.174
Days to first fruit harvest	0.130 L	-0.350
Peduncle length (cm)	0.074 N	0.232
Fruit length (cm)	-0.249 M	-0.484*
Fruit width (cm)	0.110 L	0.483*
Fruit weight (g)	-0.009 N	-0.696*
Total number of fruits per plant	0.000 N	0.643*
No. of marketable fruits per plant	-0.280 M	0.522*
Seed length to breadth ratio	-2.281 H	-0.303
No. of seed per fruit	0.015 L	-0.433*
100 seed weight (g)	0.001 L	0.651*

* Significant at 5% level

Table 3. Oil Content and and fatty acid composition of bottle gourd seed.

Name	Fatty Acid Composition							
	Oil content (%)	16:0 Palmitic	18:0 Stearic	18:1 Oleic	18:2 Linoleic	18:3 Linolenic	20:0 Arachidic	Oleic and linoleic acid ratio
IC546146	19.94	12.4	7.0	7.0	73.1	0.1	0.3	0.10
IC546148	24.96	14.0	7.5	6.6	71.3	0.2	0.4	0.09
IC546149	22.49	17.9	9.2	7.4	64.8	0.2	0.5	0.11
IC546150	25.21	15.3	10.2	10.6	63.3	0.1	0.5	0.17
IC546160	21.72	15.8	8.2	7.5	67.8	0.2	0.5	0.11
IC546168	21.76	16.3	8.5	8.0	66.6	0.2	0.4	0.12
IC546170	27.01	14.6	7.5	7.4	70.1	0.1	0.3	0.11
IC546175	26.28	13.4	6.7	6.1	73.3	0.1	0.3	0.08
IC546178	25.79	14.0	7.5	7.3	70.6	0.2	0.4	0.10
IC546181	27.10	14.7	7.0	6.4	71.3	0.2	0.4	0.09
IC446592	28.00	13.6	7.8	9.7	68.4	0.1	0.4	0.14
IC446593	25.99	12.8	8.0	8.3	70.4	0.1	0.4	0.12
IC446598	18.64	16.0	9.2	10.1	63.6	0.5	0.6	0.16
IC446599	20.44	14.6	8.8	8.4	67.6	0.2	0.4	0.12
IC446600	25.99	12.6	8.3	7.4	71.1	0.2	0.4	0.10
IC570400	18.31	15.3	8.9	8.4	66.8	0.2	0.4	0.13
IC570480	22.14	13.1	8.0	6.1	72.2	0.2	0.4	0.08
IC570504	22.60	14.3	7.6	7.0	70.5	0.2	0.4	0.10
Arka Bahar	20.93	14.0	6.6	6.3	72.0	0.2	0.9	0.09
Biogaurav	24.97	13.8	9.5	7.1	68.9	0.2	0.5	0.10
Pusa Naveen	21.68	14.4	6.7	5.6	72.7	0.2	0.4	0.08
Swathi	27.83	13.2	9.2	8.9	68.2	0.1	0.4	0.13

Figure 1. Map showing the collection sites of bottle gourd germplasm from the state of Andhra Pradesh of Peninsular India

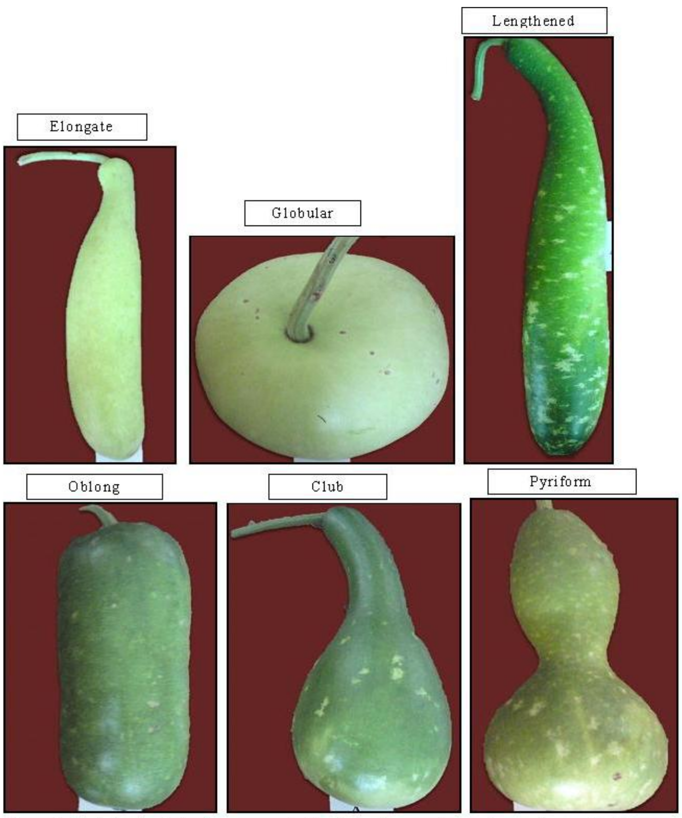

Figure 2. Variation in fruit shapes recorded in the bottle gourd germplasm characterized

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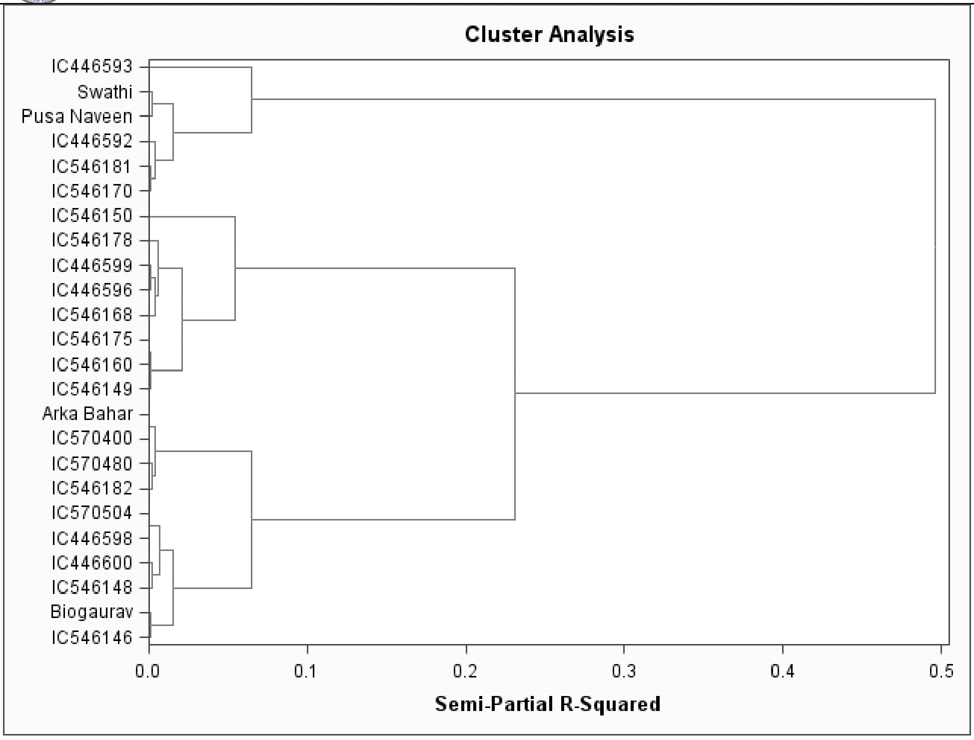

Figure 3. Multivariate cluster analysis of the bottle gourd germplasm lines using Ward's minimum method