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Selecting parental lines among monoecious cucumber genotypes for future breeding aiming at downy mildew disease tolerance

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Abstract
Cucumber production is threatened by heavy incidence of downy mildew disease during rainy and autumn-winter seasons in the tropics. Identifying and deploying tolerant germplasm are required for cucumber geneticists to develop promising hybrid/variety against this disease. The experiment was undertaken to study different components of genetic variability for 20 growth and yield component traits employing 20 genotypes as well as to screen genotypes against downy mildew disease in Randomized Block Design with three replications. Three genotypes Debstar, Samrat 7 star, and Pusa Barkha showed resistant reactions with less than 10% leaf infection under open field condition. High magnitude of GCV and heritability coupled with high genetic advance as percentage of mean was observed for vine length, intermodal length, nodal position of female flower, number of fruits per plant, average fruit weight, sex ratio, seeds per fruit, 100 seed weight, total sugar content, vitamin C content and percent disease index (PDI) of downy mildew, and thus selection may be rewarding for further improvement of these traits. Number of fruits per plant, seeds per fruit and fruit weight were identified as important selection indices. Based on the divergence study all the twenty genotypes grouped into 5 clusters. The pattern of distribution of genotypes from diverse geographical region into different clusters was random. The character PDI of downy mildew disease contributed maximum towards the divergence followed by vitamin C content, seeds per fruit, fruit yield per and fruit diameter. Based on multivariate analysis and average performance for fruit yield per plant and downy mildew disease severity, six genotypes Debstar, Samrat 7 star, Pusa Barkha, PCUC-8, Swarna Ageti, and Pusa Uday could be identified as potential donors for utilization in future breeding of cucumber to develop high yielding disease resistant hybrid/variety.

Keywords: Cucumber, variability, diversity, principal component analysis, downy mildew tolerance

Introduction
Cucumber (*Cucumis sativus* L.) is an important vegetable crop grown throughout the world under tropical and sub-tropical climates. It is the second most widely cultivated cucurbit after watermelon and fourth most important vegetable crop after tomato, cabbage and onion (Tatilioglee, 1993) [66]. The centre of origin of cucumber is considered to be India (De candolle, 1882; Bisht *et al*., 2004; Sebastian *et al*., 2010) [14, 7, 60]. The possible progenitor of cucumber *Cucumis hardwickii* R. (Alef) is found in the foothills of the Himalayas.
Cucumber is a warm season crop grown commercially throughout India in areas extending from plains to higher altitude including river beds. In India it covers an area of about 0.08 million hectares with an annual production of about 1.14 million tonnes with the productivity of 14.64 million tonnes per hectare which is considerably low compared to world average of 31.7 tonnes per hectare (Anonymous, 2017) [2].
A good salad dressing without cucumber is impossible. Fruits are rich source of Ca, P, Fe and ascorbic acid, thiamine, riboflavin and niacin (Singh *et al*., 2001) [65]. In spite of its high acceptability among growers and consumers, and wide range of available genetic variability, India is still lagging behind to attain the optimum productivity in cucumber owing to use of local unimproved cultivars and heavy infestations of insect-pest and diseases particularly fungal and viral diseases which inflict economic loss (Gupta *et al*., 2014; Yousuf and Dar, 2016) [27, 75]. Among fungal diseases, downy mildew *Pseudoperonospora cubensis* (Berk. and Curt.) Rostow, once established in a region, can spread rapidly, causing significant loss of

fruit quality and yield during rainy and autumn-winter seasons in the Gangetic plains of eastern India (Anonymous, 2013-14) [1]. Reports from Egypt stated that downy mildew can cause yield losses up to 70-100% in cucumber (El-Hafaz *et al*., 1990) [21]. Cucurbit downy mildew pathogen is an obligate parasite and, with the rare exception of oospore production, can only survive and reproduce on living host tissue (Bains and Jhooty, 1976) [4]. The pathogen generally thrives well in warm and humid regions of tropical and sub-tropical climates. Leaf wetness of 8-10 hours is critical for infection to occur, with sporangia requiring free moisture to germinate, but a temperature of 15°C determines the rate of disease (Cohen, 1977; Anonymous, 2013-14) [12, 1]. The current control relies mainly on multiple fungicide applications that exert selection pressure on the fungus, increasing the risk of the development of fungicide resistance in the pathogen population (Holmes *et al*., 2006) [30]. Moreover, frequent use of fungicides can be harmful to the environment and detrimental to natural enemies (Kookana *et al*., 1998; Kibria *et al*., 2010; Komarek *et al*., 2010) [41, 37, 40]. The satisfactory control of disease may be achieved with the application of certain fungicides but complete and environmental safer protection from the disease through host plant resistance is more preferred and effective option. Therefore, the use of resistant cultivars could provide farmers with economic and environmentally sound management strategies for downy mildew control (Metwally and Rakha, 2015) [47]. Many comprehensive studies were conducted over multiple years for screening of cucumber accessions against resistance to downy mildew in North Carolina (Wehner and Shetty, 1997) [71], Poland (Klosinska *et al*., 2010; Call *et al*., 2012) [38, 9], Egypt (Metwally and Rakha, 2015) [47], India (Dhillon *et al*., 1999; Ranjan *et al*., 2015; Pal *et al*., 2017) [18, 56, 50]. Neykov and Dobrev (1987) [48] reported that the most resistant cultivars of cucumber against downy mildew disease were of Asian origin, mostly from Japan followed by India and China.

Monoecious is the predominant sex form in cucumber and they are suitable for growing under open field conditions as compared to gynoecious cultivars which require protected condition. Therefore, much concentrated efforts are necessary to improve its yield, quality and host plant resistance against downy mildew among monoecious genotypes. Hence, evaluation of the potentialities of the indigenous germplasm is essential because promise for further improvement programme depends on the genetic diversity of the crop. The magnitude of heritable and more particularly its genetic components, is clearly the most important aspect of the genetic constitution of the breeding material which has a close bearing on its response to selection. Again selection of one trait invariably affects a number of associated traits which evokes the necessity in findings out the interrelationship of various yield components both among themselves and with yield. The proper choice of parents based on their genetic divergence is a prerequisite in any sound breeding programme. Of the various sex forms available in cucumber, monoecious and gynoecious are important from hybrid production point of view (Kohli and Vikram, 2005) [39]. Hybrids involving monoecious parent could also be exploited for commercial cultivation as advocated by Jat *et al*. (2016) [31]. In India very less number of hybrids developed so far in cucumber *vis-à-vis* the developed hybrids are not so popular among the farmers of eastern India with regard to fruit quality. Therefore, the development of hybrids using potential monoecious parental lines would be very useful for open field cultivation generally undertaken by small and marginal growers who are unable to afford high cost of protected structure, with respect to improve yield, earliness and disease tolerance.

Keeping in view the importance of the study, the present investigation was undertaken to determine genetic variability components for important growth, fruit and disease severity traits influencing yield as well as interrelationship among the characters and their direct and indirect effects on fruit yield to identify important selection indices and to assess genetic divergence among monoecious cucumber genotypes through multivariate analysis to identify potential donors for future breeding.

Materials and Methods

Plant material and field growing

The present investigation was conducted at research field of All India Coordinated Research Project on Vegetable Crops, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India situated at 23.5°N latitude, 89°E longitude at an elevation of 9.75 m above the mean sea level. Field experiment was carried out employing 20 genotypes of monoecious cucumber genotypes collected from different places of India.

The experimental site was thoroughly prepared by repeated ploughing with power tiller followed by harrowing. During final land preparation 15 tonnes of FYM/ha was applied as basal along with Recommended dose of inorganic fertilizers @ 120:60:50 (N: P: K) kg/ha was applied. Urea, single super phosphate and muriate of potash were used as source of N, P and K, respectively. The entire quantity of phosphorus, potassium and half of nitrogen were applied during final land preparation. Remaining N fertilizer was applied in two equal splits (one quarter each) at 30 DAS and at flowering, respectively. After completion of layout, the genotypes were assigned to separate plot (5m × 2m area) in each replication by using random numbers. Before sowing the seeds of each genotype were soaked in water for overnight for getting uniform germination. Then two numbers of presoaked seeds were sown on 10th May, 2016 in each hill by dibbling method following randomized complete block design with three replications. Spacing was maintained at 2m × 1m accommodating 10 plants per plot. All the management practices such as irrigation, weeding, trellising, disease and pest management as scheduled for cultivation were followed as per Chattopadhyay *et al.* (2007) [10].

Data recording

Observations were recorded on five randomly selected equally competitive plants in each genotype in each replication for different quantitative characters vine length, number of primary branches, internodal length, days to first female flower appearance, nodal position of first female flower, days to first harvest, fruit length, fruit diameter, number of fruit per plant, fruit weight, fruit yield per plant, sex ratio (M/F), seeds per fruit, 100 seed weight, total soluble solids (TSS), total sugar content, vitamin C content and dry matter content. Severity of downy mildew was recorded periodically from all the plants from each plot in each replication during early morning. The nature of the spread of the disease was studied through visual observation from the initiation of the disease at seven days interval till final harvest (105 days after sowing, DAS). Susceptible plants usually showed significant downy mildew symptoms within 20 days after sowing. Genotypes were screened on a 0 to 9 scale (Jenkins and Wehner, 1983) [32] based on the percentage of

symptomatic leaf area (0=0%, 1 =1–5%, 2 = 6-10%, 3 = 11-20%, 4 = 21-30%, 5 = 31-50%, 6 = 51-65%, 7 = 66-80%, 8 = 81-99%, and 9 = 100%). The classification of accessions into resistant and susceptible groups is somewhat subjective but in keeping with previous studies (Dhillon *et al*., 2007) [16] accessions were grouped into five categories on the basis of percent disease index (PDI): 0-10% - resistant (R); 11-20% - moderately resistant (MR); 21-30% - moderately susceptible (MS); 31- 40% - susceptible (S); > 40% - highly susceptible (HS). The percent disease index (PDI) was calculated by the following formula given by Wheeler (1969) [72].

$$PDI = \frac{N_1 \times 1 + N_2 \times 2 + N_3 \times 3 + N_4 \times 4 + N_5 \times 5 + N_6 \times 6 + N_7 \times 7 + N_8 \times 8 + N_9 \times 9}{\text{Total number of observed leaves} \times \text{Maximum grade}} \times 100$$

Where N_1 to N_9 represents total number of leaves falling under 1-9 scales, respectively.

Statistical analyses

Analysis of variance was carried out as per the procedure given by Panse and Sukhatme (1985) [53]. The genotypic (GCV) and phenotypic (PCV) coefficients of variations were computed according to Burton (1952) [8]. Heritability in broad sense and genetic advance (GA), as percent of mean were estimated as per Hanson *et al*. (1956) [28]. Phenotypic and genotypic correlation coefficients between different variables were calculated according to Johnson *et al*. (1955) [33]. Path coefficient analysis was used to partition the genotypic correlation into components of direct and indirect effects as per Dewey and Lu (1959) [15]. The D^2 statistic was used to assess genotype genetic divergence for quantitative traits by following Mahalanobis, 1936 [46] D^2 method. Tocher's method as described by Rao (1952) [57] was used for the grouping of the populations. Hierarchical cluster analysis had been done with those same genotypes in order to observe the degree of association according to their characteristics that was expressed in dendrogram following Ward's (1963) [70] method. Principal component analysis (PCA), as the method of identifying the factor dimension of the data, was used to summarize varietal information in a reduced number of factors for selection of the best performing genotype(s).

Results and discussion

Disease severity of cucumber genotypes

Cucumber genotypes were screened against downy mildew disease at final harvest (105 DAS) based on the percentage of infected leaf from total population of 10 plants using the scale. Some of the researchers (Dhillon *et al*., 1999; Ranjan *et al*., 2015; Jat *et al*., 2016) [18, 56, 31] have relied upon screening techniques of cucumber genotypes against downy mildew disease on the basis of PDI at different DAS. Reactions of different genotypes, in terms of severity values of downy mildew differed at final harvest. Normally, under the Gangetic plains of eastern India, the symptom appears at early vegetative stage, which is approximately 20 to 25 days after sowing. Typical leaf symptom was first observed at 20-25 DAS in genotypes Panipat Local, Dharwad Green, Kalyanpur Green, Swarna Sheetal, Swarna Poorna, WBC-40, Naogra Green and Super Long Green in varying degrees which escalated with increase in days while there was no symptom in genotypes Debstar, Samrat 7 star, Pusa Barkha, PCUC-8, Swarna Ageti, and Pusa Uday during this period (Table 1). Three genotypes Debstar, Samrat 7 star, and Pusa Barkha showed resistant reactions with less than 10% leaves infection, five genotypes PCUC-8, Poinsett, Swarna Ageti, Pusa Uday and Rajmata were categorized as moderately resistant with 11-20% leaf infection, three genotypes Punjab Naveen, Summer Queen and Mednipur Local exhibited moderately susceptible reactions with 21-30% leaf infection, seven genotypes Dharwad Green, Kalyanpur Green, Swarna Sheetal, Swarna Poorna, WBC-40, Naogra Green and Super Long Green were categorized as susceptible with 31-40% leaves infection, whereas the genotype Panipat Local exhibited more than 40% leaves infection and was categorized as highly resistant at final harvest. The disease severity value was lowest in Debstar (8.10%) followed by Pusa Barkha (9.18%) and Samrat 7 star (9.24%), and highest in Panipat Local (41.45%).

Downy mildew was observed to cause disease on cucumber as early as the 19th century, but it was not until the mid-1980s that it occurred on an economically significant scale (Colucci *et al*., 2006) [13]. The disease reached epidemic levels in cucumber grown in Central-Eastern Europe during 1985. It became a serious problem in the USA starting in 2004 (Colucci *et al*., 2006) [13]. Now-a-days, yearly downy mildew epidemics threaten cucumber production in up to 80 countries, causing significant economic losses (Lebeda and Urban, 2004; Colucci *et al*., 2006) [44, 13].

Disease resistance can be broadly defined as the host's ability to suppress or inhibit a pathogen's activity (Ton *et al*., 2006) [67]. The resistance exhibited by various genotypes may be due to presence of resistance genes. Three recessive resistance genes were reported in studies using different plant materials by Doruchowski and Lakowska-Ryk (1992) [20], who designated them as *dm1*, *dm2* and *dm3*. On the other hand, Vliet and Meysing (1974) [69] reported that resistance in cv. 'Poinsett' was determined by one recessive gene (*dm*). The single-gene resistance was later confirmed by Fanourakis and Simon (1987) [23]. The original source of resistance varies over downy mildew inheritance studies. Some studies evaluated resistance sources from PI 197087 (India) while other studies evaluated resistance from P.R. 40 (China) and other germplasm sources. There are at least three genes for resistance to downy mildew, coming from P.R. 40, PI 197087 and PI 197088. The combination of two different sources should provide either better resistance or more durable resistance. Reuveni *et al*. (1990) [59] observed that individuals with high peroxidase activity were resistant to *P. cubensis* when inoculated.

The most resistant genotypes in this study might exhibit a hypersensitive response (HR) with production of small chlorotic flecks and sparse sporulation, while the most susceptible genotypes were highly chlorotic and necrotic. The HR type resistance was first described by Barnes and Epps (1954) [5] in cucumber PI 197087, from a single resistance gene *dm1*.

In previous study, Ranjan *et al*. (2015) [56] found the variety Pusa Uday to be susceptible unlike our study in different environment. Host resistance responses can be affected by environmental factors and multiple pathotypes and races (Lebeda and Widrlechner, 2003) [45]. The reason for these highly different and heterogeneous responses to this fungal pathogen is not obvious; nevertheless it is assumed that specific pathogenic strains for certain hosts may have evolved only in certain parts of the world and are not found elsewhere or these hosts may only be susceptible where a number of

environmental factors such as temperature, humidity, rainfall and inoculum movement by wind coincide (Cohen, 1977) [12]. This indicates that it is necessary to evaluate different accessions in local conditions against the *P. cubensis*.

Table 1: Downy mildew disease severity and its reaction under open field condition in 20 genotypes of cucumber

Genotypes	PDI of downy mildew (%) at harvest	Reaction of downy mildew
Raima Cucumber	29.72	MS
Debstar	8.10	R
Dharwad Green	39.23	S
Poinsett	17.87	MR
Pusa Barkha	9.18	R
Pusa Uday	12.85	MR
Panipat Local	41.45	HS
PCUC-8	13.74	MR
Punjab Naveen	27.54	MS
Kalyanpur Green	36.89	S
Swarna Sheetal	30.37	S
Swarna Poorna	34.82	S
Swarna Ageti	14.10	MR
Summer Queen	25.18	MS
WBC-40	38.52	S
Naogra Green	37.67	S
Super Long Green	35.57	S
Mednipur Local	24.67	MS
Samrat 7 Star	9.24	R
Rajmata	16.42	MR
Mean	27.36	1.04
C.V.	2.25	8.59
S.E.m (±)	0.36	0.05
C.D. 5%	1.02	0.15

R= Resistant; MR= Moderately resistant; MS= Moderately susceptible; S= Susceptible; HS= Highly susceptible

Genetic variability and heritability for different characters

Estimates for the co-efficient of genotypic and phenotypic variation (GCV and PCV respectively), heritability in broad sense (h^2), and genetic advance (GA) as per cent of mean for different characters are presented in Table 2.

Genotypic and phenotypic coefficient of variation is simple measure of variability, commonly used for the assessment of variability. The relative value of these types of coefficients gives an idea about the magnitude of variability present in a population. Close estimates of GCV and PCV were recorded for all the characters. In general, PCV was marginally higher than the corresponding GCV indicated less influence of environment in the expression of the characters under study. It also implies that contribution towards final phenotypic expression of these characters is mostly by genetic makeup of these genotypes rather than the environmental factors and suggested that the selection could be effective on the basis of phenotypic alone with equal probability of success. The GCV ranged from 5.90% (days to first male flower appearance) to 34.69% (percent disease index of downy mildew), while PCV ranged from 6.01% (days to first male flower appearance) to 34.77% (percent disease index of downy mildew). High proportion of GCV to PCV is desirable in selection process because it depicts that the traits are much under the genetic control rather than the environment. High magnitude of GCV as well as PCV values (>20.00%) were recorded for the traits *viz*., seeds per fruit (20.40% and 20.71%, respectively), vitamin C content (20.26 and 20.40, respectively) and percent disease index of downy mildew (34.69% and 34.77%, respectively).The higher the GCV, the more will be the chance for exploitation of that particular character in a selection programme. Gaikwad *et al.* (2011) [25] also reported same estimates for percent disease index of downy mildew. Moderate GCV and PCV values (10.00-20.00%) were recorded for vine length (15.74% and 16.04%, respectively), internodal length (12.68% and 12.91%, respectively), nodal position of female flower (17.95% and 19.88%, respectively), sex ratio (18.04% and 18.72%, respectively), 100 seed weight (12.99% and 13.43%, respectively), total soluble solids (10.12% and 11.19%, respectively), total sugar content (13.32% and 13.67%, respectively), dry matter content (12.97% and 14.45%, respectively) and fruit yield per plant (15.20% and 17.46%, respectively), suggested existence of considerable variability in the population. Selection for these traits may also be given the importance for improvement programme. Similar results were reported by Faruk *et al*. (2010) [24], Gaikwad *et al*. (2011) [25] and Bhawana *et al*. (2010) [6] in cucumber. Low GCV and PCV values (<10.00%) were recorded for days to first male flower appearance (5.90% and 6.01%, respectively), days to first female flower appearance (8.19% and 8.28%, respectively), days to first harvest (6.89% and 7.47%, respectively), fruit length (7.54% and 7.91%, respectively), fruit diameter (8.51% and 9.24%, respectively) and fruit weight (6.56% and 6.82%, respectively). Selection based on these characters will be less effective for hybridization programme which agreed well with the observations of Arunkumar *et al*. (2011) [3] and Shah *et al*. (2018) [62] for days to first male and female flower appearance, days to first harvest; Golabadi *et al*. (2012) [26] for fruit length. In this study the proportion of genetic contribution to the overall phenotypic expression of most of the traits was very high ranging from 71.79% in number of primary branches to 99.77% in percent disease index of downy mildew. Therefore, their use as important discriminatory variable for cucumber classification study seems relatively reliable.

With the help of GCV alone, it is not possible to determine the amount of variation that is heritable. The heritable portion of the variation was determined with the aid of heritability

estimates. Heritability suggests the relative role of genetic factors in expression of phenotypes and also acts as an index of transmissibility of a particular trait to its off-springs. However, the knowledge of heritability alone does not help to formulating concrete breeding programme, genetic advance along with heritability help to ascertain the possible genetic control for any particular trait. The genetic advance provides the knowledge about expected gain for a particular character after selection. According to Johnson *et al*. (1955) [33] genetic advance as per cent of mean depends upon selection differential, phenotypic coefficient of variation and heritability ratio. The nature and extent of the inherent ability of a genotype for a character is an important parameter determining the extent of improvement of any crop species.

Heritability estimate provide the information regarding the amount of transmissible genetic variation to total variation and determine genetic improvement and response to selection. Heritability estimate along with genetic advance are normally more useful in predicting the gain under selection by separating out environmental influence from total variability than that of heritability alone (Burton, 1952) [8].

In the present investigation the heritability estimates ranged from 51.00% (Number of primary branches) to 99.90% (Percent disease index of downy mildew). High magnitude of heritability (more than 80%) was recorded for all the characters under the study except number of primary branches, dry matter content and fruit yield per plant. The high estimates of heritability in the quantitative characters has been found to be useful from plant breeders' view point as this would enable him/her to base the selection on the phenotypic performance. Yadav *et al.* (2009) [74] reported high heritability estimates for fruit length and weight. High heritability estimates for node at first female flower, days to first female flower opening, days to first harvest, number of fruits per plant and fruit diameter were supported the findings of Dhiman and Prakash (2005) [19], Arunkumar *et al*. (2011) [3] and Veena *et al*. (2012) [68]. Moderate heritability estimates were found in number of primary branches, dry matter content and fruit yield per plant.

Genetic advance (GA) as percentage of mean was observed moderate to high for all the characters under the study and ranged from 11.88% (number of primary branches) to 71.32% (PDI of downy mildew). High GA as per cent of mean (> 20%) was recorded for vine length, intermodal length, nodal position of female flower, number of fruits per plant, sex ratio, seeds per fruit, 100 seed weight, total sugar content, vitamin C content, dry matter content, percent disease index of downy mildew and fruit yield per plant. High GA as per cent of mean for these characters was reported by Veena *et al*. (2012) [68] and Chikezie *et al*. (2016) [11] in cucumber. Moderate GA as per cent of mean (10-20 %) was recorded for the characters number of primary branches, days to first male flower appearance, days to female flower appearance, days to first harvest, fruit length, fruit diameter, fruit weight and total soluble solids.

Heritability estimates along with genetic advance are more useful than the heritability value alone for selecting the best individual. It has been suggested that characters with high heritability coupled with high genetic advance would respond to selection better than those with high heritability and low genetic advance (Johnson *et al*., 1955) [33]. It is not always true that high heritability for a character yields high genetic advance. If the heritability estimates are mainly due to additive gene effects, then it would be associated with high genetic advance and if they were due to non-additive gene effects, genetic advance would be low (Panse, 1957) [52].

High heritability coupled with high genetic advance as percentage of mean was observed for vine length, intermodal length, nodal position of female flower, number of fruits per plant, average fruit weight, sex ratio, seeds per fruit, 100 seed weight, total sugar content, vitamin C content and percent disease index of downy mildew indicated that these characters were mainly controlled by additive gene effects (Panse, 1957) [52] and thus selection may be rewarding for the further improvement of these traits. These findings corroborated with earlier workers [Shah *et al*. (2018) [62] for vine length, nodal position of female flowers, number of fruits per plant; Kandasamy (2017) [36] for 100 seed weight; Pal *et al.* (2016) [51] for PDI of downy mildew]. Moderate heritability coupled with high genetic advance was observed for dry matter content and fruit yield per plant but not as efficiently as first group of characters. High heritability with moderate genetic advance as percentage of mean was observed for days to first male flower appearance, days to first female flower appearance, days to first harvest, fruit length, fruit diameter, fruit weight and total soluble solids. Moderate heritability with moderate genetic advance was observed for number of primary branches.

Table 2: Mean, range and estimates of genetic parameters of twenty cucumber genotypes

Character	Mean	Range	GCV (%)	PCV (%)	GCV: PCV	Heritability in b.s. (%)	Genetic advance as (%) of mean
Vine length (cm)	180.75	131.47 - 233.21	15.74	16.04	98.13	96.00	31.82
Number of primary branches	5.16	3.93 - 5.87	8.04	11.20	71.79	51.00	11.88
Internodal length (cm)	7.59	5.93 - 9.51	12.68	12.91	98.22	96.00	25.65
Days to first male flower appearance	32.63	29.20 - 35.87	5.90	6.01	98.17	96.00	11.93
Days to first female flower appearance	39.34	33.67 - 45.13	8.19	8.28	98.91	98.00	16.67
Nodal position of female flower	5.66	3.60 - 7.27	13.99	15.27	91.62	84.00	26.42
Days to first harvest	47.53	41.67 - 53.00	6.89	7.47	92.24	85.00	13.09
Fruit length (cm)	14.77	13.36 - 17.10	7.54	7.91	95.32	91.00	14.79
Fruit diameter (cm)	3.97	3.32 - 4.54	8.51	9.24	92.10	85.00	16.15
Number of fruits per plant	7.16	4.16 - 9.37	17.95	19.88	90.29	81.00	33.37
Fruit weight (g)	145.85	123.51 - 159.84	6.56	6.82	96.19	93.00	13.00
Sex ratio (M/F)	4.61	3.62 - 6.43	18.04	18.72	96.37	93.00	35.80
Seeds per fruit	192.83	131.80 - 250.48	20.40	20.71	98.50	97.00	41.39
100 seed weight (g)	2.21	1.53 - 2.64	12.99	13.43	96.72	94.00	25.89
Total soluble solids contents (°brix)	3.52	2.80 - 4.20	10.12	11.19	90.44	82.00	18.84
Total sugar content (%)	2.39	1.67 -2.87	13.32	13.67	97.44	95.00	26.73
Vitamin C content (mg/100 g)	4.44	2.98 - 6.11	20.26	20.40	99.31	99.00	41.43
Dry matter content (%)	3.64	2.79 - 4.95	12.97	14.45	89.76	80.00	23.97

Percent disease index of downy mildew (%)	27.36	12.10 - 41.45	34.69	34.77	99.77	99.90	71.32
Fruit yield per plant (kg)	1.04	0.64 - 1.32	15.20	17.46	87.06	76.00	27.27

GCV = Genotypic coefficient of variation; PCV = Phenotypic coefficient of variation

Character association

Correlation coefficient analysis measures the mutual relationship between various plant characters and determines the component characters on which selection can be based for improvement in yield. In general, yield is a quantitative trait governed by several genes and depends on several other contributing traits, which are under monogenic, and oligogenic governance. Improvement of yield is the ultimate objective of crop improvement programmes. Yield can be effectively improved by applying selection pressure on yield attributing traits, which are associated with yield. Correlation between two characters is due to either pleiotropic genes or genetic linkage of genes governing them. In the present study correlation coefficient among various characters have been estimated and presented in Table 3.

Fruit yield per plant showed highly positive significant correlation with vine length (rg=0.628**, rp=0.526**), number of primary branches (rg=0.958**, rp=0.637), number of fruits per plant (rg=0.920** rp= 0.929**), seeds per fruit (rg=0.688**, rp=0.599**) and 100 seed weight (rg=0.486**, rp=0.425). Fruit yield per plant also exhibited significant negative correlation with days to first harvest (rg=-0.585**, rp= -0.529**), fruit length (rg=-0.440*, rp=-336*), fruit diameter (rg=-0.293), fruit weight (rg=-0.239*), sex ratio (rg=-0.939, rp=-0.804**), total sugar content (rg=-0.247*), vitamin C content (rg=-0.271*) and percent disease index of downy mildew (rg=-0.944**, rp=-0.822**). Positive correlation of vine length, number of primary branches and number of fruits per plant is logical as increase in these parameters leads to increased fruit yield per plant. These findings were in agreement with that of Kumari *et al*. (2018) [43] for vine length and number of fruits per plant; Chikezie *et al.* (2016) [11] for number of primary branches per plant. Similarly, a valid explanation for negative correlation of sex ratio (M/F) as increase in male flowers certainly reduces the number of female flowers which ultimately reduces fruit yield per plant and PDI of downy mildew is indication of disease incidence percentage. Less severity of downy mildew disease causes to increase fruit yield per plant.

It was also observed that vine length had significant and positive correlation with number of primary branches, days to female flower appearance, nodal position of female flower, number of fruits per plant, seeds per fruit and 100 seed weight. Vine length also showed significant and negative correlation with internodal length, fruit diameter, sex ratio and PDI of downy mildew. Similarly, characters like number of fruits per plant, seeds per fruit and 100 seed weight had significant positive correlation with number of primary branches per plant. Positive and significant correlation of number of fruits per plant with seeds per fruit and 100 seed weight and negative significant association of this character with fruit weight, days to first harvest, fruit length and diameter was noticed. Seeds per fruit were significantly and positively correlated with 100 seed weight and vitamin C content and significant negative correlation with total sugar content. Vitamin C content had significant positive association with 100 seed weight, total soluble solids, total sugar content, dry matter content and PDI of downy mildew.

From the above discussion, it became evident that fruit yield per plant can be increased through increase in component traits like vine length, number of primary branches, number of fruits per plant, seeds per fruit and 100 seed weight.

Path coefficient analysis was based on correlation coefficients using fruit yield per plant as the dependent factor (effect) and fix other quantitative characters as independent factor (causes). The concept of path co-efficient analysis was originally developed by Wright (1921) [73], but the technique was first used for plant selection by Dewey and Lu (1959) [15]. Genotypic pathway associations of different characters of 20 cucumber genotypes are presented in Table 3.

The correlation coefficient of each independent quantitative character was partitioned into direct and indirect effect towards fruit yield. As the residual effect was very low (0.0332), it is therefore, indicated that the number of characters chosen for the study were very much appropriate for determination of fruit yield in cucumber. 11out of 19 characters showed positive direct effects towards fruit yield per plant. Number of fruits per plant imparted the highest positive direct effect (1.115) on fruit yield followed by seeds per fruit (0.505), fruit weight (0.416), days to first female flower appearance (0.357) and fruit length (0.201). Number of fruits per plant exhibited high positive direct effect along with significant positive correlation with yield indicating the importance of this character in indirect selection for yield. This result corroborated with earlier findings of Arunkumar *et al.* (2011) [3] and Kumar *et al.* (2013) [42], Sharma *et al*. (2018a) [64] and Kumari *et al*. (2018) [43].

High indirect effects of the different characters were also noticed through vine length, number of primary branches, days to first harvesting and number of primary branches per plant, indicating the need for emphasis on these traits during selections for yield improvement.

Though fruit weight and fruit length imparted the high and moderate positive direct effects, respectively on fruit yield per plant, but negative correlation coefficient with fruit yield indicated that the negative indirect effects are the cause of manifestation of the correlation. Therefore, a restricted selection model maybe employed to nullify the undesirable indirect effects in order to make the use of positive direct effects of fruit weight and fruit length in cucumber improvement programmes.

Vine length was significantly correlated with fruit yield per plant in positive direction but had negative direct effect on fruit yield per plant indicated the indirect effect of other component characters were the main cause for the production of such correlation coefficient. In this circumstance, other causal factors with high indirect effect should be considered during selection for yield improvement in cucumber.

From the study of character association ship, combining both correlation and path co-efficient, the characters, namely, number of fruits per plant, seeds per fruit, fruit weight, days to first female flower appearance and fruit length were the most important selection criteria as they exerted high positive direct effects on fruit yield per plant.

Table 3: Genotypic and Phenotypic correlations and direct effects of nineteen characters at phenotypic level on marketable fruit yield/plant

Character	rg with yield/plant	rp with yield/plant	Direct effect on yield/plant
Vine length (cm)	0.628**	0.527**	-0.063
Number of primary branches	0.958**	0.637**	0.033
Internodal length (cm)	-0.111	-0.089	0.010
Days to first male flower appearance	0.055	0.015	-0.032
Days to first female flower appearance	-0.476**	-0.418**	0.062
Nodal position of female flower	-0.062	-0.062	-0.009
Days to first harvest	-0.585**	-0.529**	-0.010
Fruit length (cm)	-0.440**	-0.336**	0.016
Fruit diameter (cm)	-0.293*	-0.237	-0.030
Number of fruits per plant	0.920**	0.929**	1.125
Fruit weight (g)	-0.239*	-0.195	0.451
Sex ratio (M/F)	-0.939**	-0.804**	-0.004
Seeds per fruit	0.688**	0.599**	0.090
100 seed weight (g)	0.486**	0.425**	-0.019
Total soluble solids content (°brix)	-0.020	-0.063	0.027
Total sugar content (%)	-0.247	-0.178	0.012
Vitamin C content (mg/100 g)	-0.271	-0.237	-0.027
Dry matter content (%)	-0.004	0.009	-0.020
Percent disease index of downy mildew (%)	-0.944**	-0.822**	-0.026

*, **significant at P≤0.05 or P≤0.01, respectively.
[a]rg = Genotypic correlation coefficient.
[b]rp= Phenotypic correlation coefficient.
[c]Residual effect= 0.0534

Genetic diversity of genotypes through multivariate analysis

The D^2 Statistic model is used to determine the divergence among population in terms of generalized group distance developed by Mahalanobis (1936) [46]. It has been widely used in Psychometry and anthropometry for classificatory purpose. Rao (1952) [57] suggested the application of this technique for assessment of genetic diversity in Plant Breeding. Multivariate analysis is a powerful tool in qualifying the degree of divergence between biological populations (genetic distance) and to assess the relative contribution of different components to the total divergence. Although, Mahalanobis's generalized distance as a measure of genetic distance occupy a unique place in plant breeding yet, as it happens in biology, several problems under the influence of random unpredictable changes due to environment, evade the direct grip of the concept well proven is more exact fields like mathematical components. In general, genetic divergence plays important role, because hybrids between genotypes of diverse origin generally display a greater heterosis and throw more recombinants than those between closely related parents. Increasing the genetic distance at first heterosis increased. However, further increase in genetic divergence causes to reduce heterosis. Hence, for hybrid seed production the breeders must be selected the parents with a moderate genetic distance (Olfati *et al.*, 2014) [49].

It suggests the measuring of the genetic distance through multivariate analysis over environment, to fortify its reliability. Genetic divergence of cucumber using Mahalanobis's statistics was earlier studied by several workers (Kumar *et al.*, 2013; Hasan *et al.*, 2015; Shah *et al*., 2018; Sharma *et al*., 2018b) [42, 29, 63].

The present study aimed at analyzing the genetic divergence of 20 genotypes employing 20 quantitative characters. Based on the degree of divergence (D^2 values) between any two genotypes a logical grouping of the genotypes with low D^2 value could be arrived at by Tocher's method as suggested by Rao (1952) [57]. Based on the determination of divergence all the twenty genotypes could be meaningfully grouped into 5 clusters (Table 4). Among the all five clusters, maximum genotypes present in Cluster-I, possessing six genotypes followed by cluster-II, which had five genotypes. Rest of the clusters had three genotypes in each. In general, the pattern of distribution of genotypes from diverse geographical region into different clusters was random. It might be due to free and frequent exchange of genetic materials among the farmers and breeders of different regions. Differential selection pressure according to regional preference also produced greater uniformity in the germplasm. The absence of relationship between genetic diversity and geographical distance indicates that forces other than geographical origin such as exchange of genetic stock, genetic drift, spontaneous mutation, natural and artificial selection are responsible for genetic diversity. Therefore, the selection of genotypes for hybridization should be based on genetic divergence rather than geographic diversity. Similar results were obtained by Prasad *et al*. (2001) [54]; Rao *et al*. (2003) [58] and Kumar *et al*. (2013) [42] in cucumber. Environmental influence on the composition of cluster was also recorded earlier in okra (Seth *et al*., 2016) [61].

The intra- and inter-cluster distance represents the index of genetic diversity among clusters as given in the Table 5. The intra and inter-cluster distance among 20 genotypes revealed that highest intra cluster distance observed in Cluster-I (1496.41), whereas cluster-III exhibited lowest intra cluster distance (797.50). Therefore, it was evident that the genotypes in this cluster are less variable. According to D^2 values, highest inter cluster distances were witnessed between Cluster-I and Cluster-IV (13235.86), followed by Cluster I and V (11651.69), Cluster III and IV (6898.17). Lowest inter cluster distances were observed between Cluster-IV and Cluster-V (1997.95), signifying close relationship among the genotypes of this group.Hence, intermating between the genotypes included in these clusters was expected to give transgressive segregates in the advanced generation. Kalloo *et al*. (1980) [35] suggested that the crosses between selected varieties from widely separated clusters were most likely to give desirable recombinants.

Cluster means of twenty characters revealed that significant variability present among the genotypes chosen for study. Cluster means of each cluster is depiction of mean value of

particular characters for genotypes present in that cluster. The cluster means for the twenty characters studied in cucumber genotypes revealed considerable differences among all the clusters. Cluster wise mean and over all cluster mean for the characters studied were presented in Table 6.

From the present data, it is evident that vine length was recorded maximum in Cluster-I (193.21 cm) and minimum in Cluster-II (169.62 cm). Number of primary branches per plant was recorded maximum in Cluster-I (5.43) and minimum in Cluster-III (4.67). Lowest internodal length was in Cluster-V (6.71cm) while maximum in Cluster- II (8.33cm). Nodal position of female flower was reported to be minimum in Cluster-I (5.29 cm) and maximum in Cluster-II (5.97).

Phonological characters which represents earliness like days to first male flower appearance, days to first female flower appearance and days to first harvest having low values fall in Cluster-V (31.18) and Cluster-I (38.10) and Cluster I (46.28) respectively, whereas, cluster-III (33.29), Cluster-IV (41.29) and Cluster-IV (48.67) had the genotypes which exhibited lateness in first male and female flower appearance and first harvest respectively. The Cluster-III had the maximum fruit length (15.59 cm), whereas, Cluster-II had the minimum phenotypic values for this character (14.12 cm). Maximum Fruit diameter was recorded in Cluster-III (4.44 cm) and minimum was recorded in Cluster-IV (3.61cm). Number of fruits per plant was recorded maximum in Cluster-I (7.63) and minimum in Cluster-III (6.07). The genotypes of Cluster- III had maximum fruit weight (154.65 g), whereas genotypes of Cluster-IV (141.53 g) had minimum values for this trait. Lower sex ratio (M/F) was observed in Cluster-I (4.25) and highest in Cluster-III (5.27). Seeds per fruit were highest in Cluster-III (205.31) and minimum in Cluster-II (179.75). Maximum 100 seed weight was observed in Cluster-I (2.29 g) and lowest was recorded in Cluster-II and IV (2.13).

Total soluble solids were found to be highest in Cluster-III (3.83 °brix) and minimum was in Cluster- V (3.33 °brix). With respect to total sugar content, Cluster-III (2.65) found to be highest and Cluster-II (2.22) found to be lowest. Vitamin C content was found to be maximum in Cluster-III (5.30 mg/100 g) and minimum was in Cluster-II (3.79 mg/100 g). Most contrasting genotypes for quality traits like TSS, total sugar and vitamin C present in Cluster-II and Cluster-III and members from these clusters are utilised for development of bi-parental population in order to dissect the genetic loci responsible for these quality traits. Maximum dry matter content was in Cluster-I (3.90 g) and minimum was in Cluster-V (3.32 g). It has been observed that genotypes in Cluster-III proved to be most susceptible to Downy mildew as they have exhibited highest percent disease index values (30.43%), whereas Cluster-I processed some contrasting tolerant genotypes with less percent disease index (21.58%). It could be advised to hybridise the plant between these two clusters in order to develop the mapping populations to identify QTLs responsible for the tolerance of Downy mildew. Fruit yield per plant was highest in Cluster-I (1.10 kg) and minimum in Cluster-III (0.94 kg). Parents from highly contrasting clusters are generally recommended for combinational breeding to transfer one or few mono/oligo genes from donor parent to recipient parent besides development of mapping populations. Parents with similar cluster means are utilised for identification of transgressive segregants by accumulation of favourable genes from both the parents as a must combine well with each other, and should preferably be genetically diverse.

Relative contribution of various characters under the study was presented in Table 6. The top five characters which contributed most towards the genetic divergence were PDI of downy mildew disease (65.79%) followed by vitamin C content (19.47%), seeds per fruit (4.21%), fruit yield per plant (3.68%) and fruit diameter (3.6%). These characters may be used in selecting genetically diverse parents for hybridization programme to exploit either maximum heterosis or to execute efficient selection in the segregating generation.

The PCA was performed to obtain a simplified view of the relationship between the characters PDI of downy mildew, and vitamin C content which explained 99.95% contribution towards divergence, and variable loadings for components PC1 (PDI of downy mildew) and PC2 (vitamin C content) were determined (Table 7). These components were chosen because their eigenvalues exceeded 1.0 and explained 99.95% of total variance. The first component (PC1) explained 96.31% of total accounted for variance in which a decrease in PDI of downy mildew leads to increase in vitamin C content (Table 8). The second component (PC2) explained an additional 3.64% of the variance in which an increase in vitamin C content was associated with increase in PDI of downy mildew. The PCA was also used to determine relationships among okra genotypes of Indian origin (Seth *et al.*, 2016; Ramgiry *et al*., 2017) [61, 55]. There are no clear guidelines to determine the importance of a trait coefficient for each principal component. Johnson and Wichern (1988) [34] regard a coefficient greater than half of the coefficient, divided by the square root of the standard deviation of the eigenvalue of the respective principal component, as significant.

In further study of dendrogram following Ward's (1963) [70] method by using squared Euclidean distance, it became clearly evident that there was high diversity among the cucumber genotypes along with strong relationships among the genotypes (Figure 1).

Accessions in close proximity are perceived as being similar in PCA; accessions that are further apart are more diverse (Figure 2). The differences observed in the data, and summarized in the PCA, indicated accessions Debstar, Swarna Ageti, Samrat 7 Star, Raima Cucumber, Summer Queen, Rajmata, PCUC-8, Mednipur Local, Panipat Local, and Poinsett were quantitatively dissimilar from others. The remainder of genotypes had similar features forming a separate cluster. From the plot of PC1 *vs*. PC2 selection may be refined considering 2 principal components, with Debstar being the best performing cultivar having optimum combination of all variables including downy mildew disease tolerance, followed by genotypes Samrat 7 Star, PCUC-8, and Swarna Ageti and can be used as improved genetic material for disease resistant breeding against downy mildew.

The expression of heterosis over mid parents (H) depends on the difference in allele frequency (y) of the parents and dominance effect (D) at various loci, i.e. H = Dy2 (Falconer, 1981) [22]. Therefore, some level of dominance and genetic diversity are necessary for the expression of heterosis. A crossing programme involving parents selected on the basis of genetic divergence may likely to produce transgressive segregates. Therefore, the choice of diverse parents with good combining ability is prerequisite for efficient hybridization programme. However, there seems to be an optimal level of diversity, beyond which heterosis does not increase or may even decrease due to unfavourable interaction of co-adopted gene complexes or physiological incompatibility (Dhillon *et al*., 2004) [17].

Keeping the genetic diversity and *per se* performance of genotypes for fruit yield, and downy mildew disease severity traits, 6 monoecious genotypes Debstar, Samrat 7 star, Pusa Barkha, PCUC-8, Swarna Ageti, and Pusa Uday were identified as good candidates for utilization in future breeding programme in cucumber to produce heterotic hybrids or recombinants in the segregating generations.

Table 4: Cluster classification of 20 genotypes of cucumber

Clusters with the number of genotypes in parentheses	Name of genotype/Source of collection
Cluster-I (6)*	Pusa Barkha (IARI, New Delhi), Super Long Green (West Bengal), Raima Cucumber (West Bengal), Rajamata (West Bengal), Poinsett (NSC, New Delhi), PCUC-8 (Pantnagar)
Cluster-II (5)	Pusa Uday (IARI, New Delhi), Swarna Ageti (Ranchi, Jharkhand), Swarna Sheetal (Ranchi, Jharkhand), Punjab Naveen (Punjab), Kalyanpur Green (Uttar Pradesh)
Cluster-III (3)	Summer Queen (West Bengal), Mednipur Local (West Bengal), Panipat Local (Haryana)
Cluster-IV (3)	Samrat 7 Star (West Bengal), Swarna Poorna (Ranchi, Jharkhand), Dharwad Green (Karnataka)
Cluster-V (3)	Naogra Green (West Bengal), Debstar (West Bengal), WBC-40 (West Bengal)

*Figures in parentheses indicate number of genotypes

Table 5: Average intra (bold) and inter cluster D^2 values for five clusters of 20 genotypes of cucumber

Clusters	I	II	III	IV	V
I	1496.41	5478.11	3587.33	13235.86	11651.69
II		1434.12	3214.97	3284.05	3700.19
III			797.50	6898.17	4351.81
IV				1059.39	1997.95
V					962.62

*Bold diagonal values indicate intra cluster distance, rest of the values show the inter cluster distances.

Table 6: Cluster means of 20 characters of cucumber genotypes

Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	% contribution towards divergence
Vine length (cm)	193.21	169.62	183.10	170.46	182.35	0.01
Number of primary Branches	5.43	5.19	4.67	5.00	5.20	0.01
Internodal length (cm)	7.41	8.33	7.68	7.49	6.71	0.01
Days to first male flower appearance	32.30	33.19	33.29	33.16	31.18	0.01
Days to first female flower appearance	38.10	40.33	38.62	41.29	38.93	0.01
Nodal position of female flower	5.29	5.97	5.93	5.42	5.87	0.01
Days to first harvest	46.28	48.60	47.00	48.67	47.67	0.01
Fruit length (cm)	15.19	14.12	15.59	14.57	14.37	0.01
Fruit diameter (cm)	4.08	3.95	4.44	3.61	3.65	3.16
Number of fruits per plant	7.63	7.54	6.07	7.05	6.79	0.01
Fruit weight (g)	145.30	141.57	154.65	141.53	149.58	0.53
Sex ratio (M/F)	4.25	4.42	5.27	4.44	5.14	0.53
Seeds per fruit	200.80	179.75	205.31	191.63	187.45	4.21
100 seed weight (g)	2.29	2.13	2.21	2.13	2.28	1.05
Total soluble solids content (°brix)	3.75	3.38	3.62	3.39	3.33	0.01
Total sugar content (%)	2.45	2.22	2.65	2.46	2.23	1.58
Vitamin C content (mg/100 g)	4.70	3.79	5.30	4.01	4.57	19.47
Dry matter content (%)	3.90	3.48	3.85	3.48	3.32	0.01
Percent disease index of downy mildew (%)	21.58	29.75	30.43	29.76	29.43	65.79
Fruit yield per plant (kg)	1.10	1.06	0.94	1.00	1.02	3.68

Table 7: Results of principal component analysis (PCA) for quantitative characters contributing to divergence

Principal component (PC)	Eigenvalue (%)	% Variance	% Cumulative variance
Eigenvalues and variance accounted for (%) by PCA based on correlation matrix			
PC_1	1593.32	96.31	96.31
PC_2	60.282	03.64	99.95

Table 8: Contribution of diverse traits in the principal components of cucumber

Variables	PC_1	PC_2
Factor loadings due to PCs with eigenvalues greater than 1		
PDI of downy mildew (%)	-0.1399	0.9888
Vitamin C content (mg/100 g)	0.0043	0.0498

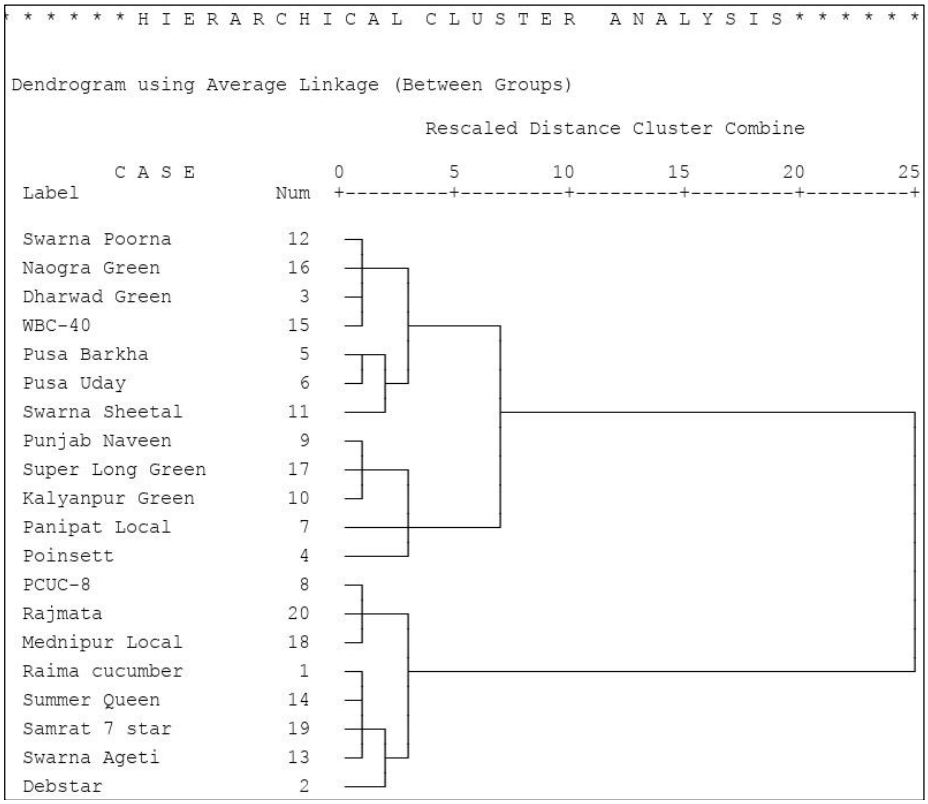

Fig 1: Dendrogram of genotypes of cucumber following Ward's method. Genotypes are in left most column

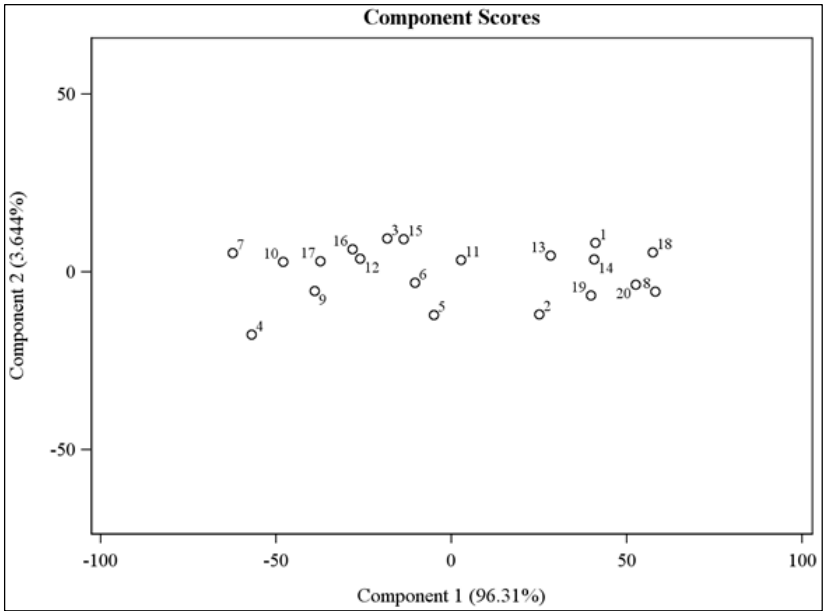

Fig 2: Scatter diagram of regression factor scores for the first and second components as determined by principal component analysis. Points in diagram closest to the intersection of 0 on the X- and Y-axes indicate similarity. Outliers on the X-axis that is 2 = Debstar, 13 = Swarna Ageti, 19 = Samrat 7 Star, 1 = Raima Cucumber, 14 = Summer Queen, 20 = Rajmata, 8 = PCUC-8, 18 = Mednipur Local, 7 = Panipat Local, and 4 = Poinsett indicate diversity. Number correspond to the name of genotype, see Figure 1.

Conclusion

Wide genetic variability was observed for 20 quantitative traits under study. The insight of variability present in a gene pool of a crop species is of utmost importance to plant breeding programme. Positive association with high direct effects for number of fruits per plant, seeds per fruit, fruit weight, days to first female flower appearance and fruit length was observed with fruit yield per plant hence, these traits may be directly attributed for the improvement of fruit yield in cucumber. The cluster pattern of the genotypes showed non-parallelism between geographic and genetic diversity. Maximum genetic divergence was recorded for PDI of downy mildew disease followed by vitamin C content, seeds per fruit, fruit yield per plant and fruit diameter. The probability of obtaining better segregates and recombinants is expected when 6 monoecious cucumber genotypes Debstar, Samrat 7 star, Pusa Barkha, PCUC-8, Swarna Ageti, and Pusa Uday can be used as donor parents.

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