Acta Scientific Agriculture (ISSN: 2581-365X)

Volume 2 Issue 9 September 2018

Research Article

Studies on Hybridization and Genetic Diversity in Cucumber (*Cucumis sativus* L.)

C Kipgen and VK Khanna*

School of Crop Improvement, College of Post-Graduate Studies, Central Agricultural University, Umiam, Meghalaya, India

***Corresponding Author:** VK Khanna, School of Crop Improvement, College of Post-Graduate Studies, Central Agricultural University, Umiam, Meghalaya, India.

Received: August 03, 2018; **Published:** August 09, 2018

Abstract

Crossability of various accessions of cucumber on the basis of pollen development and fruit set was studied along with genetic diversity by SSR markers. There was a non-significant correlation between fruit set and percent viable pollen. In all selfings and the crosses, pollen germination increased from 1 hour to 4 hours after pollination. Pollen germination had a highly significant correlation with fruit set. On selfing, the fruit set was positively correlated with faster pollen tube growth whereas it was not so in the case of crosses. There was a positive correlation between pollen tube growth and fruit set. Nineteen SSR primers were selected to assess genetic diversity of 35 accessions of cucumber. The polymorphic SSR primers generated a total of 33 alleles which ranged from 2 to 4 with an average of 2.53 per locus. Cluster analysis performed based on Bray Curtis similarity matrix showed that highest dissimilarity was found in cluster I which comprised of only one accession, IIHR-76 which is distinct from the rest of the accessions. The value of Bray-Curtis similarity coefficient ranged from 33 to 100 with the average value of 65. In the principal component axes, the first component PCA plot explained 22% variation, whereas the second and the third component explained 15.1% and 13.4% variation, respectively. The grouping obtained through PCA was comparable to Bray Curtis similarity matrix cluster analysis.

Keywords: Crossability; Cucumis sativus; Pollen Tube; Fruit Set; SSR

Cucumber (*Cucumis sativus* L.) is one of the most economically important vegetables crops grown worldwide. With the exception of *Cucumis sativus* var. *hardwickii*, almost all cucumbers are day neutral, requiring no definite photoperiod for flowering. On the other hand, high temperatures and long days tend to promote maleness. Under certain environmental conditions, mainly under stress conditions, gynoecious and hermaphroditic lines generate a number of staminate flowers which differ from line to line [1]. According to Frankel and Galun [2] short day conditions in melon and cucumber plants induced the early appearance of pistillate flowers and increased their number.

The assessment of pollen viability is important in artificial pollination and breeding experiments [3]. For successful fertilization to occur there should be components of pollen performance which includes pollen produced in a flower, pollen morphological homogeneity, pollen germination, pollen tube growth and pollen competition [4-6]. The first requirement needed must be an availability of an adequate source of viable and compatible pollen. Secondly, an effective transfer of pollen when stigmas are receptive. Thirdly, pollen tubes must grow down the style and enter the ovule when embryo sacks have matured. And finally, double fertilization and subsequent growth of embryo and endosperm must occur to provide the necessary stimulus for fruit development [4]. Intraspecific hybrids are an important source of variability in breeding programs. The knowledge of the intraspecific compatibility as well as of the direction of crossing is extremely important for success in hybrid breeding programs [7].

Genetic diversity analysis facilitates utilization of genetic resources for conservation of germplasm and the development of breeding programs [8]. Cucumber has a very narrow gene pool which limits development of new cucumber varieties by cross-breeding [9]. The knowledge of genetic diversity of cucumber germplasm is extremely essential for cucumber breeders to produce new cucumber varieties with higher yield and better quality [10]. This paper reports pollen germination and pollen tube behavior in relation to crossability between various accessions of Cucumis sativus and seed set and genetic diversity using SSR primers.

Materials and Methods

The 35 accessions used were VRCU-379, IIHR-341, IIHR-B, PCUC-08, Kalyanpur long, VRCU-102-09-02, IIHR-76, K-90, PCUC-09, SPP-88, VRCU-03, SPP-44, Phule Subhangi, VRCU-93, IIHR-127, VRCU-52, VRCUH-19, Swarna Sheetal, IIHR-82, IIHR-177, CH-122, IIHR-339, SPP-98, VRCU-12-06, Swarna Agheti, VRCU-12-02, IIHR-337, DC-54, IIHR-404 and accessions of M-1, M-2, NG-1, NG-2, MZR, ASM which were collected from different locations in North Eastern Region. Five parents were selected for crossability studies. They were used for one way crossing and so the total number of selfing and crossing were 15 (Table 2). For each cross at least fifteen flowers were randomly selected from plants. Pollinations were performed from April to June, 2016 and care was taken to avoid any damage to the stigma. The styles were collected 1 to 4 hours (as fertilization takes place within that period) after hand pollination and fixed immediately in 1:3 glacial acetic acid-ethyl alcohol for at least 24 hours and then preserved in 70 per cent alcohol till further use. For pollen germination and pollen tube growth observations, the pollinated flowers were gently rinsed in distilled water and pistils were separated from the flowers after which they were kept in a drop of 1N HCl for 10 minutes. They were again rinsed in distilled water and stained in 1 percent aniline blue [11,12]. The time required for staining was 10 - 20 seconds depending on the thickness of the style and the stage of penetration of the pollen tube in the stigma. After staining, the pistils were destained for 20 - 24 hours in a 1:1:1 mixture of 40% acetic acid: orthophosphoric acid: distilled water. The pistils were then rinsed in distilled water and mounted in pure lactic acid and studied under the microscope. The pollen grains and pollen tubes stained deep blue.

Genomic DNA from cucumber leaves was isolated using CTAB method of Doyle and Doyle [13]. Young actively growing leaves of 15 - 20 days old plants were collected and used for DNA extraction. The quantification of DNA were done by staining DNA with ethidium bromide after electrophoresis in 0.8% agarose gel at 80 V for 1 hour in TBE buffer (0.04M Tris borate, 0.001M EDTA, pH 8.0) using known DNA concentration standards. Molecular weights of bands were estimated by using 100 bp for SSR. The homology of bands was based on the distance of migration in the gel. SSR amplicons obtained from each entry were resolved as multiple and a single band, respectively on the agarose system and the pair-wise correlation matrix was developed on the normalized data set to find out the relationship among 35 accessions of cucumber. Dendrogram was constructed by hierarchical cluster analysis (group-average linking) using the Bray-Curtis resemblance matrix (Clarke, 1999). The correlation matrix was subjected to 'Eigen' vectors analysis, following which the principle components were extracted. The first three most important PCA were used to construct a three dimensional plot of the accessions. The PCA, hierarchical cluster, Bray-Curtis resemblance matrix and correlation matrix analyses presented in this thesis was computed using PRIMER-E v6.1.9 software (Primer-E Ltd, Plymouth, UK). The raw data matrix was used to calculate correlations between variables. The correlation matrix was subjected to 'Eigen' vectors analyses (Clarke, 1999) following which the principle coordinates were extracted using the 'Projection' module in NTSYS-PC. The first three most important PCoA were used to construct a three dimensional plot of the accessions. These computations were performed using the programme NTSYS-PC (ver. 2. 02 j) [14].

Results and Discussion

Pollen fertility

The pollen fertility of all the accessions under this study revealed that the maximum fertile stained pollen was recorded in M-1 (92.88 ± 0.28) whereas the lowest percentage of fertile pollen recorded was in DC-54 (86.37 ± 0.30) followed by IIHR-404 (86.72 ± 0.39) (Table 1). However, the average percent pollen fertility did not show too much difference among the accessions. Hedhly., *et al.* [6] reported that temperature is an important factor affecting pollen performance during the progamic phase, from pollination to fertilization.

Pollen germination

Maximum pollen germination after 1 hour of pollination was recorded in selfing of VRCU-03 (63.21%) and the least pollen germination was recorded in selfing of DC-54 (41.90%). The results show that there was a constant increase in pollen germination from 1 hour to 4 hours in all the parents. After 1 hour of pollination, on selfing, maximum pollen germination was recorded in VRCU-03 (63.21%), followed by M-1 (61.15%), IIHR-404 (45.59%), M-2 (44.78%), and DC-54 (41.90%). After 4 hours of pollination on self-

ing, maximum pollen germination was recorded in M-1 (83.58%) and the least was recorded in DC-54 (56.84%) (Figure 1).

Sl. No.	Parents	Pollen fertility (%)	Fruit set (%)
1	M-1	92.88 ± 0.28	79
2	VRCU-03	91.79 ± 0.13	64
3	DC-54	86.37 ± 0.30	69
4	M-2	88.54 ± 0.78	46
5	IIHR-404	86.72 ± 0.39	55

Table 1: Pollen fertility in the parents in cucumber.

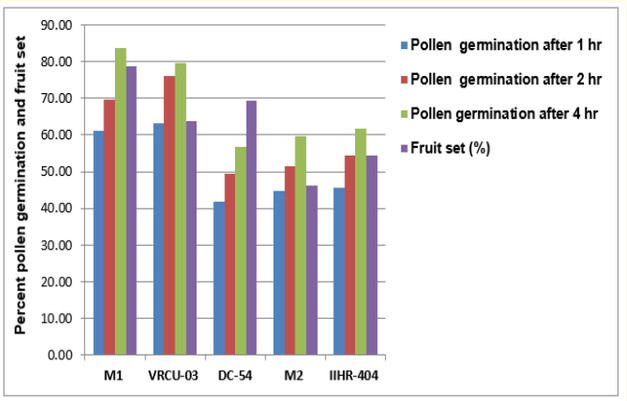

Figure 1: Pollen germination at different time intervals and percent fruit set in selfing.

In crosses between the parents, the maximum pollen germination was recorded in M-2 X IIHR-404 (62.99%) after 1 hour of pollination and the least was found in DC-54 X M-2 (43.93%) after 1 hour of pollination. After 4 hours of pollination, maximum pollen germination was recorded in VRCU-03 X IIHR-404 (83.63 ± %) and the least was recorded in DC-54 X M-2 (67.47%) (Figure 2).

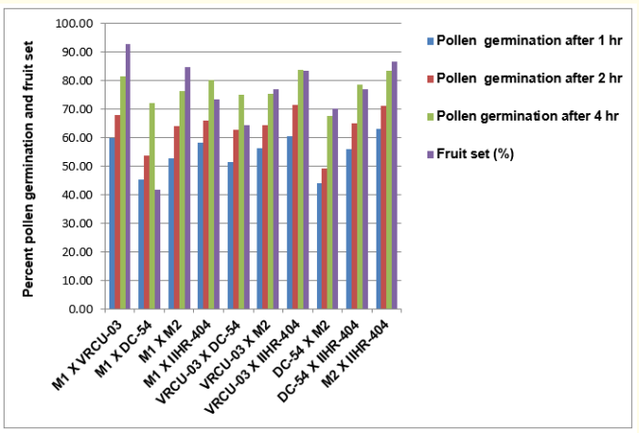

Figure 2: Pollen germination at different time intervals and percent fruit set in crosses.

Fruit set on selfing was maximum in M-1 (79%) where pollen germination was also maximum i.e. 83.58% and it was second best in DC-54 (69%) where the pollen germination was the least i.e. 56.84% after 4 hours of pollination (Figure 1). In crosses, M-1 X VRCU-03 showed the highest fruit set (93%) which showed 81.32% pollen germination after 4 hours of pollination (Figure 2). The highest pollen germination after 4 hours of pollination was recorded in the cross VRCU-03 X IIHR-404 (83.63) where fruit set was also quite good i.e. 83%. The least pollen germination was seen in DC-54 X M-2 (67.47%) after four hours of pollination and the fruit set in this cross was also next to the least i.e. 70%. In all selfings and the crosses, pollen germination increased from 1 hour to 4 hours after pollination.

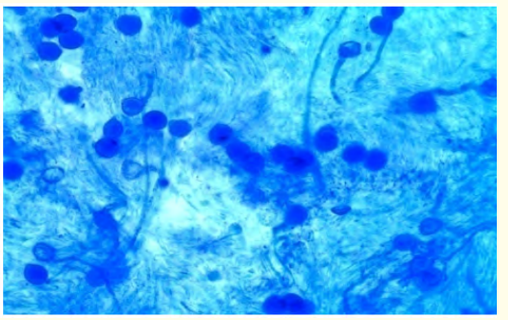

Figure 3: Pollen germination and pollen tube growth in M-2 X IIHR-404, after 2 hours of pollination (10 X).

In one case the pollen germination in the selfing after 4 hours of pollination (83.58%) was nearly the same as in a cross i.e. VRCU-03 X IIHR-404 (83.63%) but in general, pollen germination was more in the crosses as compared to the selfings. Similar results could be seen for fruit set. Aloni., *et al.* [15] in bell pepper established a high correlation between *in vitro* pollen germination and fruit set under high temperature conditions. This suggests that pollen germination could be a useful tool for testing cultivar tolerance to high temperature.

Pollen tube growth

The emergence of pollen tubes on the stigma and the style was seen after different time intervals on selfing and in crosses. After penetrating the stigma hairs, the tubes grew through these hairs into the style. Because of the irregularity in pollen grain germination, and poor staining of pollen tubes it was very difficult to measure the pollen tube length in the thick tissues at the base of the style.

At 1 hour after pollination, a number of pollen grains had germinated and the tubes had penetrated into the stigma hairs. Some of the pollen tubes had just started their growth in the hairs and a few had already entered the transmitting tissue of the style. After 1 hour of selfing, the maximum pollen tube growth was observed in M-1 (232.33 µm) and the minimum in M-2 (118.67 µm) (Figures 4 and 6). In crosses, maximum pollen tube growth was observed in VRCU-03 X M-2 (284 µm) and the minimum was observed in M-1 X DC-54 (161.10 µm) (Figure 5).

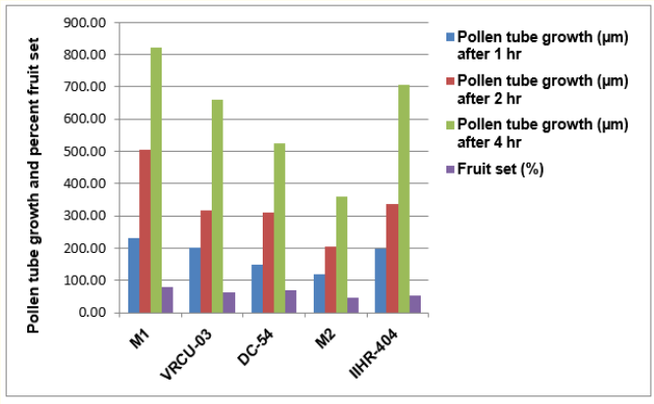

Figure 4: Pollen tube growth at different time intervals and percent fruit set in selfing.

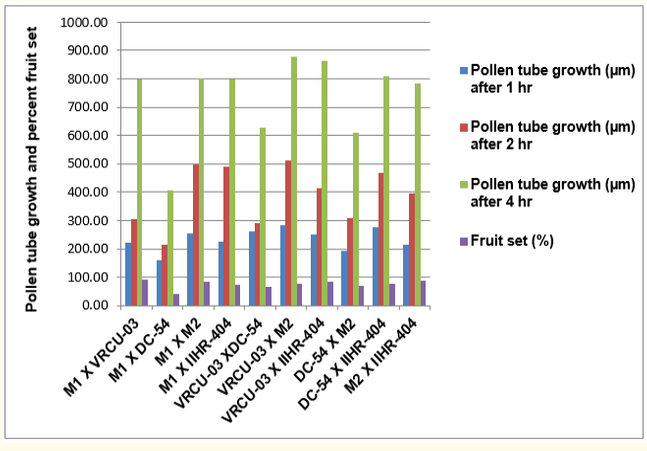

Figure 5: Pollen tube growth at different time intervals and percent fruit set in crosses.

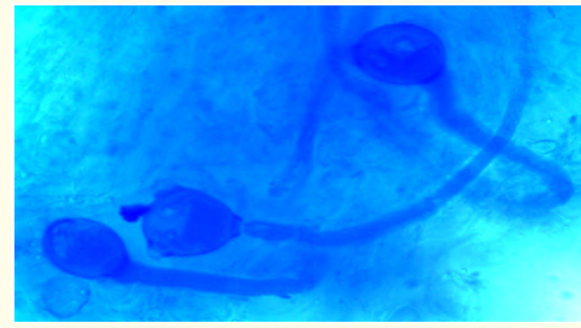

Figure 6: Pollen tube growth in M-2 on selfing, after 4 hours of pollination (40X).

After 2 hours of pollination, there was a greater differentiation and elongation in the pollen tubes whereas some had just started to elongate and all intermediate stages were observed. After 4 hours of pollination, very long pollen tubes were recorded and since the styles were quite long, the pollen tubes could not be traced up to the base of the style as their staining became faint as they worked their way towards the ovary. On selfing, maximum pollen tube growth was observed in M-1 (823.67 µm) and the minimum was observed in M-2 (361.33 µm). In crosses, similarly maximum pollen tube growth was observed in VRCU-03 X M-2 (879.33 µm) and minimum pollen tube growth was observed in M-1 X DC-54 (407.96 µm). So, the results were quite similar at 1 hour, 2 hours and 4 hours after pollination.

On selfing, the fruit set was positively correlated with faster pollen tube growth whereas it was not so in the case of crosses. The maximum pollen tube was recorded in the case of VRCU-03 X M-2 (879.33 µm), where the fruit set was not maximum (77%). But the least pollen tube growth was in M-1 X DC-54 (407.96 µm) and in this case even the fruit set was the least i.e. 42%.

In some crosses pollen tube growth was more than in the selfings (VRCU-03 X M-2; VRCU-03 X IIHR-404) as well the fruit set, for example in M1 X VRCU-03, M-2 X IIHR-404, M-1 X M-2 and VRCU-03 X IIHR-404. Debbarama., *et al.* [16] and Kharkongar., *et al.* [17] also reported that pollen tube growth was positively correlated with fruit set.

Fruit set

Table 2 shows that the maximum fruit set was obtained in crosses as compared to selfings. Among the selfing, the maximum fruit set was obtained in M-1 (79%) and the least in M-2 (46%) (Figure 7 and 8). When the parents were crossed, the maximum fruit set was obtained in M-1 X VRCU-03 (93%) and the least fruit set was obtained in M-1 X DC-54 (42%) (Figure 9 and 10). In general, fruit set was less in selfings.

Sl. No.	Parents/Crosses	Pollen germination after 4 hours (%)	Pollen tube growth after 4 hours (µm)	Fruit set (%)
1	M-1	83.58	823.67	79
2	VRCU-03	79.44	659.17	64
3	DC-54	56.84	524.00	69
4	M2	59.63	361.33	46
5	IIHR-404	61.77	705.00	55
6	M1 X VRCU-03	81.32	797.56	93
7	M1 X DC-54	72.20	407.96	42
8	M1 X M2	76.22	801.00	85
9	M1 X IIHR-404	80.04	799.33	73
10	VRCU-03 X DC-54	74.97	626.33	64
11	VRCU-03 X M2	75.31	879.33	77
12	VRCU-03 X IIHR-404	83.63	863.33	83
13	DC-54 X M2	67.47	610.85	70
14	DC-54 X IIHR-404	78.55	807.67	77
15	M2 X IIHR-404	83.24	783.00	87

Table 2: Pollen germination, pollen tube growth and percent fruit set on selfing and in crosses.

Figure 7: Fruit set of IIHR-404 after selfing.

Figure 8: Fruit set of VRCU-03 after selfing.

Figure 9: Fruit set of M-2 X IIHR-404.

Figure 10: Fruit set of M-1 X IIHR-404.

In one case the pollen germination in the selfing after 4 hours of pollination (83.58%) was nearly the same as in a cross i.e. VRCU-03 X IIHR-404 (83.63%) but in general, pollen germination was more in the crosses as compared to the selfings. Similar results could be seen for fruit set.

On selfing, the fruit set was positively correlated with faster pollen tube growth whereas it was not so in the case of crosses. The maximum pollen tube was recorded in the case of VRCU-03 X M-2 (879.33 µm), where the fruit set was not maximum (77%). But the least pollen tube growth was in M-1 X DC-54 (407.96 µm) and in this case even the fruit set was the least i.e. 42%.

In some crosses pollen tube growth was more than in the selfings (VRCU-03 X M-2; VRCU-03 X IIHR-404) as well as the fruit set, for example in M-1 X VRCU-03, M-2 X IIHR-404, M-1 X M-2 and VRCU-03 X IIHR-404 (Table 2). Le Deunff., *et al.* [18] reported that the period of female receptivity in which pollination occurs influences fruit maturation and seed set in *Cucumis sativus*. From the study, it was observed that fruit set had highly significant correlation with pollen germination and a positive correlation with pollen tube growth (Table 3). Lu., *et al.* [19] reported that the use of stigmas and pollen which are at their appropriate developmental stage ensures maximum fertilization potential resulting in the production of viable seed reflecting true crossability. El Balla [20] revealed that the percentage of fruit set increased with the increase in time after pollination and higher number of pollen tubes reached the ovules after six hours from pollination.

Crossability can act as an important ingredient for successful transfer of those desirable genes. But it is always hindered by number of factors viz. genotypic and environmental [21]. Dellaporta and Calderon-Urrea [22] reported that outcrossing in flowering plants avoids the deleterious effects of inbreeding depression and promotes heterozygosity, genetic variability, and genetic exchange, which in turn leads to the long-term survival and adaptation of a species.

Correlation studies in crosses of *Cucumis sativus*

Correlation studies on pollen germination recorded non-significant values for percent viable pollen whereas pollen germination after 2 hrs of pollination was highly correlated with pollen germination after 1 hr of pollination, and pollen germination after 4 hrs of pollination had a highly significant correlation with pollen germination after 1 and 2 hrs of pollination, respectively.

Similarly, pollen tube growth recorded non-significant correlation with percent viable pollen. Pollen tube growth after 1 hr of pollination was observed to be highly correlated with pollen germination after 1 hr, 2 hrs and 4 hrs of pollination. In the same way, pollen tube growth after 4 hrs of pollination exhibited high correlation with pollen germination after I hr, 2 hrs and 4 hrs of pollination, and also with pollen tube growth after 1 hr and 2 hrs of pollination. But pollen tube growth after 2 hrs of pollination showed correlation with pollen germination after 1 hr, 2 hrs and 4 hrs at 5% level of significance and was also highly correlated with pollen tube growth after 1 hr of pollination.

Fruit set had highly significant positive correlation with pollen germination (0.654, 0.595, and 0.639) and pollen tube growth at 1% level of significance (0.638, 0.664 and 0.830) but a negative non-significant correlation was seen between fruit set and percent viable pollen (-0.065) (Table 3).

Characters	Percent viable pollen	PG after 1 hr of pollination	PG after 2 hr of pollination	PG after 4 hr of pollination	PTG after 1 hr of pollination	PTG after 2 hr of pollination	PTG after 4 hr of pollination	Fruit set (%)
Percent viable pollen	1.000							
PG after 1 hr of pollination	0.214	1.000						
PG after 2 hr of pollination	0.199	0.979**	1.000					
PG after 4 hr of pollination	0.048	0.914**	0.896**	1.000				
PTG after 1 hr of pollination	-0.326	0.586**	0.611**	0.678**	1.000			
PTG after 2 hr of pollination	0.108	0.555*	0.523*	0.552*	0.753**	1.000		
PTG after 4 hr of pollination	-0.018	0.734**	0.704**	0.714**	0.851**	0.864**	1.000	
Fruit set (%)	-0.065	0.654**	0.595**	0.639**	0.638**	0.664**	0.830**	1.000

Table 3: Correlation studies for various characters in crosses of Cucumis sativus.

PG: Pollen Germination; PTG: Pollen Tube Growth

Note: * denotes values at 5 % level of significance; ** denotes values at 1 % level of significance.

SSR analysis

Twenty primers were used for genetic diversity analysis and 13 primers were found to be polymorphic. The polymorphic primers generated a total of 33 alleles.

The data obtained from SSR analysis were subjected to Bray Curtis similarity analysis to find out the relationship among the accessions analyzed. The value of Bray-Curtis similarity coefficient ranged from 33 to 100. Based on Bray-Curtis similarity matrix it was found that the lowest similarity coefficient was found between PCUC-09 and IIHR-76 (33), whereas (SPP-88, NG-1), (Phule Subhangi, VRCU-102-09-02), (VRCUH-19, NG-1), (VRCUH-19, SPP-88), (IIHR-177, VRCU-102-09-02), (IIHR-177, Phule Subhangi), (VRCU-12-06, VRCU-93), (Swarna Aghetil, VRCU-93), (MZR, VRCU-93), (Swarna Agheti, VRCU-12-06), (MZR, VRCU-12-06), (MZR, Swarna Agheti), (DC-54, IIHR-337), (ASM, IIHR-337) and (ASM, DC-54) showed the highest similarity coefficient (100). Most of the *Cucumis sativus* accessions showed similarity coefficient between 70 to 80%.

From the dendrogram generated using Bray Curtis analysis of the data, the genotypes were grouped into two major clusters (Figure 11). The first major cluster I comprise of only one accession, IIHR-76 and appear to be the most distinct from all others. The second major cluster II was divided into 2 sub- clusters. The sub- cluster I consisted of VRCU-03 and PCUC-09 which are highly similar to each other and sub- cluster II was further divided into 2 small sub- clusters. The small sub- cluster I comprises of K-90 and Kalyanpur long. The small sub- cluster II was further divided into 2 smaller sub- clusters. The smaller sub- cluster I comprises of IIHR-404 and IIHR-82, while the smaller sub- cluster II further showed sub- groups with 2 sub- clusters. Sub- cluster I comprises of only one accession NG-2 which has dissimilarity from the rest of genotypes in this sub- cluster. Sub- cluster II again is further divided into 2 sub- clusters. Small sub- cluster I consisted of CH-122, PCUC-08 and IIHR-341. Small sub- cluster II is further grouped into 5 sub- clusters, comprising of SPP-88, NG-1, VRCUH-19, VRCU-12-02, VRCU-52, MZR, Swarna Agheti, VRCU-12-06, VRCU-93, Phule Subhangi, VRCU-102-09-02, IIHR-177, SPP-44, VRCU-379, SPP-98, IIHR-339, M-1, Swarna Sheetal, IIHR-127, IIHR-B, DC-54, IIHR-337, ASM and M-2.

Sl. No.	Code	Sequence (5' – 3')	GC Content (%)	Alleles
1	SSR 18377	F:GCCATGGATGGAGTTTT R:TCCCTTTCTCTGTTTTCCC	45.00 45.40	2
2	EC 39	F:CCAAGTTTAAGTTATTTAGGAG R:GAAGAGGACGATAAAGATGA	31.80 38.00	4
3	EC 11	F:TCTTCGCAGTCACCATTTC R:CCTTCCTCTGTTTCTGTTCC	47.30 47.60	2
4	UWO 44015	F:AAAACCCCCACAAATTCCTC R:GACGCTTCTCCTTCTCGATG	45.00 52.30	2
5	EC 18	F:TGCCATTTCATCGACTCTTC R:GCATTTCTGCTGTGGCTTAG	45.00 47.60	2
6	SSR 01115	F:ATTCCCAATCCCAAAAAGGT R:CTCCTCCTCCAATGAGCAAG	40.00 52.30	3
7	UWO 29476	F:ATTTCGATTGGGAAAAAGGG R:GCTGGCTCCTTCACATTGTT	40.00 47.60	3
8	UWO 24560	F:AGGGGGATCTTGCCTCTAA R:AGAGTGGCTCGCCAAAAGTA	52.60 47.60	4
9	UWO 74973	F:CACCGTTGGTCTTGGAACTCT R:GGGTAAGGGGTTGTATTGTTTC	52.30 43.40	2
10	EC 34	F:GATCCCCATCATAATCACCC R:CAAAGGGCTACAATAACAAAC	50.00 36.30	2
11	SSR 19511	F:TGGCGTTGCTAATTGATTGA R:ACCCGATTCGTAAGATCGTC	40.00 47.60	2
12	UWO 15964	F:ATTGGCGATTTGCTTTCAAT R:CACCCACGTGCTGTAAAAAG	35.00 47.60	3
13	EC 54	F:TTCATCACCCCTTTTCCCTT R:AAACACGATTTCCCAACACC	45.00 42.80	2

Table 4: Forward and reverse primer sequence for polymorphic SSR primers.

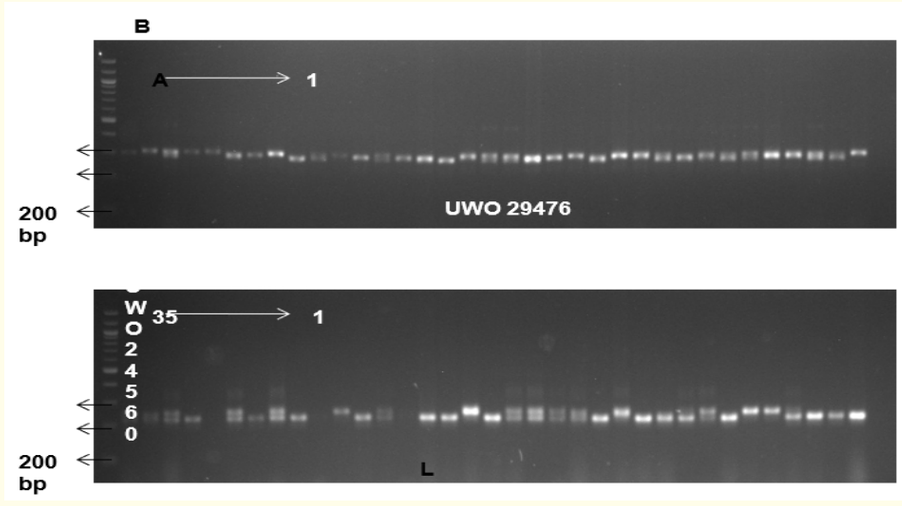

Figure 11: (A) and (B) show SSR profiles generated by UWO24560 and UWO29476 primer, respectively, on 35 accessions of Cucumis sativus. Number 1- 35 refer to genotypes mentioned in Annexure I. L indicates 100 bp ladder.

Accurate assessment of the levels and patterns of genetic diversity can be a great importance in crop breeding for diverse applications including (i) introgressing desirable genes from diverse germplasm into the available genetic base [23] and (ii) analysis of genetic variability in cultivars [24]. SSRs are appropriate for the determination of genetic diversity because they are abundant in the genome, are highly polymorphic, and reliable, and usually have a co-dominant mode of inheritance [25]. In the present study genetic diversity among 35 accessions of cucumber was assessed by using 19 SSR markers (Figures 11A and 11B). A total of 33 alleles were detected using 13 polymorphic SSR loci with the mean value of 2.53 per locus. This was similar to that reported by Innark., *et al.* [10] who analyzed genetic diversity in 38 accessions of cucumber using 20 SSR markers. It revealed 36 polymorphic alleles with an average of 2.05 alleles per primer. Pandey., *et al.* [26] assessed genetic diversity in Indian cucumber using 53 polymorphic microsatellite markers where a total of 163 amplification products were obtained and detected an average of 3.05 alleles per locus. Ning., *et al.* [27] assessed 64 accessions of Chinese melon by using 36 polymorphic SSR markers where a total of 145 alleles were detected with an average of 4.03 per SSR marker.

The pair-wise similarity matrix for all 35 accessions ranged from 33 to 100 with the average value of 65%. However, some of the accessions showed similarity value of 100. This may be due to lesser number of SSR primers used in the present study.

Cluster analysis performed based on Bray Curtis similarity matrix generated two major clusters. Cluster I comprised of only one accession (IIHR-76) which was distinct from the rest of the accessions (Figure 12). This may be due to the particular accession generating monomorphic band in almost all the primers used in the present investigation or most of the primers did not show amplification. Cluster II was divided into II A and II B sub clusters. The sub cluster II B was further divided into two small clusters comprising of four small sub grouped clusters with two to four accessions each.

In the principal component axes, the first component PCA plot explained 22% variation and second and third components explained 15.1% and 13.4% variation, respectively among the 35 accessions of cucumber (Figure 13). The grouping obtained through PCA was comparable to Bray Curtis similarity matrix cluster analysis [28-33].

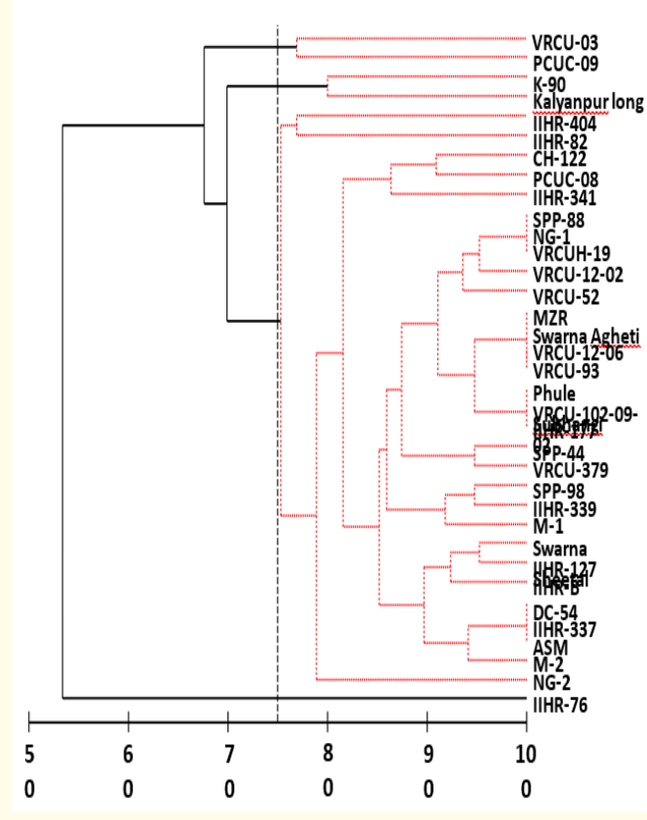

Figure 12: Dendrogram generated using Bray Curtis similarity analysis showing relationship between 35 accessions of cucumber using 19 SSR markers.

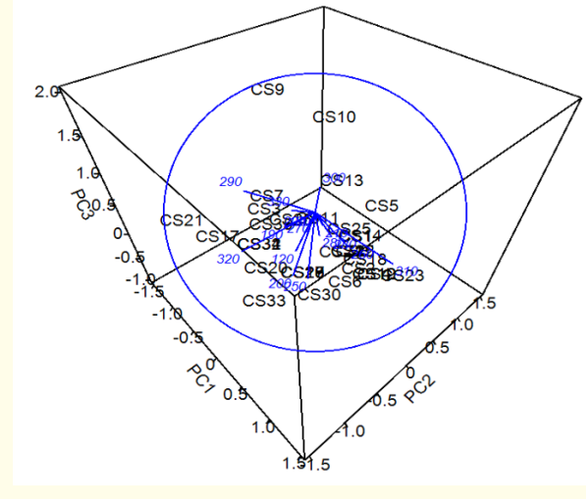

Figure 13: PCA plot depicted variability among cucumber accessions derived from the binary matrix based on presence and absence of SSR marker bands. The significant correlation between SSR marker and principal axes 1, 2 and 3 were shown as vectors indicated by blue lines

Principal component axes	Eigen values	% variation	Cumulative % variation
PC1	0.404	22.0	22.0
PC2	0.278	15.1	37.1
PC3	0.246	13.4	50.5

Table 5: Eigen values of the principal component axes and the percent variations explained by the principal components on PCA plot generated based on the variations among the SSR marker bands.

SSR marker bands	Score on PC1	Score on PC2	Score on PC3
120	0.222*	-0.366*	0.035
180	0.049	-0.071	0.070
190	-0.046	-0.237*	-0.027
200	0.168*	-0.358*	-0.306*
210	0.246*	-0.156*	0.159*
220	-0.025	-0.042	-0.007
230	0.067	0.104*	-0.300*
250	0.308*	-0.347*	-0.211*
260	0.457*	-0.143*	0.111*
270	0.093	-0.122*	0.065
280	0.065	-0.017	-0.199*
290	-0.099	-0.480*	0.445*
300	-0.051	0.093	0.169*
310	0.554*	0.241*	-0.316*
320	-0.465*	-0.375*	-0.573*
330	0.010	-0.214*	0.171*

Table 6: Eigen vectors (SSR marker bands) with coefficients in the linear combinations that make up principal components in the PCA plot.

Conclusion

Fruit set had a highly significant positive correlation with pollen germination and pollen tube growth at 1% level of significance, which means that high pollen germination and pollen tube growth are important for more fruit formation. Maximum fruit set was in the cross M1 x VRC (93%) followed by M2 x IIHR-40 (83%). So, these can be easily crossed. Cucumber has a very narrow gene pool which limits development of new cucumber varieties by cross-breeding [9]. IIHR-76 was found to be the most distinct from all others and it may be used for future breeding programmes for crop improvement.

Bibliography

1. Lower RL and Edwards MD. "Cucumber breeding". In: Bassett MJ (ed.) Breeding vegetable crops. AVI, Publishing Co. Westport, Connecticut (1986): 173-207.

2. Frankel R and Galun E. "Pollination mechanisms, Reproduction and Plant Breeding". Springer- Verlag, Berlin, Heidelberg, New York (1977).

3. Rodriguez-Riano T and Dafni A. "A new procedure to assess pollen viability". *Sexual Plant Reproduction* 12.4 (2000): 241-244.

4. Thompson M. "Flowering, pollination and fruit set". In: Webster AD and Looney NE (ed.) Cherries, Crop Physiology, Production and Uses. Wallingford, CABI Publishing (2004): 223-243.

5. Janick J and Moore NJ. "Fruit Breeding, Tree and Tropical Fruits". John Wiley and Sons, New York (1996).

6. Hedhly A., *et al*. "The effects of temperature on pollen germination, pollen tube growth and stigmatic receptivity in peach (Prunus persica L Barsch.)". *Plant Biology* 7.5 (2005): 476-483.

7. Nascimento NFF., *et al*. "Intraspecific cross-compatibility in ornamental pepper". Acta Horticulturae 1087: XXV International EUCARPIA Symposium Section Ornamentals: Crossing Borders (2015).

8. Hu J., *et al*. "Microsatellite diversity, population structure, and core collection formation in melon germplasm". *Plant Molecular Biology Reporter* 33.3 (2015): 439-447.

9. Staub JE., *et al*. "Conformity and genetic relatedness estimation in crop species having a narrow genetic base: the case of cucumber (Cucumis sativus L.)". *Plant Breeding* 124.1 (2005): 44-53.

10. Innark P., *et al*. "Evaluation of genetic diversity in cucumber (Cucumis sativus L.) germplasm using agro-economic traits and microsatellite markers". *Scientia Horticulturae* 162 (2013): 278-284.

11. Gerlach D. "A rapid safranine-crystal violet-light green staining sequence for paraffin sections of plant materials". *Stain Tech* 44.4 (1969): 210-211.

12. D'Souza L. "Staining pollen tubes in styles of cereal with cotton blue: Fixation by ethanol-lactic acid for enhanced differentiation". *Stain Tech* 47.2 (1972): 107-108.

13. Doyle JJ and Doyle JL. "Isolation of plant DNA from fresh tissues". *Focus* 12 (1990): 13-15.

14. Rohlf F J. "NTYSIS-PC, Numerical Taxonomy and Multivariate Analysis system". Ver.2.02j. Exeter Software, New York, USA (1993).

15. Aloni B., *et al*. "The effect of high temperature and high atmospheric CO2 on carbohydrate changes in bell pepper (Capsicum annuum) pollen in relation to its germination". *Physiologia Plantarum* 112.4 (2001): 505-512.

16. Debbarama C., *et al*. "Wide Hybridization and Embryo-Rescue for Crop Improvement in Capsicum". *Agrotechnology* S11 (2013): 003.

17. Kharkongar HP., *et al*. "Wide Hybridization and Embryo-Rescue for Crop Improvement in Solanum". *Agrotechnology* S11 (2013): 004.

18. Le Deunff E., *et al*. "Effect of ovular receptivity on seed set and fruit development in cucumber (Cucumis sativus L.)". *Sexual Plant Reproduction* 6.2 (1993): 139-146.

19. Lu N., *et al*. "A preliminary study on the crossability in Robinia pseudoacacia L". *Euphytica* 206.3 (2015): 555-566.

20. El Balla MMA. "Studies on floral Biology in okra (Abelmoschus esculantus (L) Moench)". M. Sc. Thesis, Submitted to University of Khartoum (1982).

21. Badiyal A., *et al*. "Interactive genotypic influence of triticale and wheat on their crossability and haploid induction under varied agroclimatic regimes". *Cereal Research Communications* 42.4 (2014): 700-709.

22. Dellaporta SL and Urrea AC. "Sex Determination in Flowering Plants". *The Plant Cell* 5.10 (1993): 1241-1251.

23. Davidar P., *et al*. "The potential for crop to wild hybridization in eggplant (Solanum melongena Solanaceae) in Southern India". *American Journal of Botany* 102.1 (2015): 1129-139.

24. Verma M., *et al*. "Genetic diversity of Indian revealed by RAPD and SSR markers". *Indian Journal of Horticulture* 69.4 (2012): 517-522.

25. Guzinski J., *et al*. "Characterization of newly developed expressed sequence tag-derived microsatellite markers revealed low genetic diversity within and low connectivity between European Saccharina latissima populations". *Journal of Applied Phycology* 28.5 (2016): 3057-3070.

26. Pandey S., *et al*. "Genetic diversity in Indian cucumber based on microsatellite and morphological markers". *Biochemical Systematics and Ecology* 51 (2013): 19-27.

27. Ning X., *et al*. "Genetic diversity among Chinese Hami melon and its relationship with melon germplasm of diverse origins revealed by microsatellite markers". *Biochemical Systematics and Ecology* 57 (2014): 432-438.

28. Ainsworth C. "Boys and girls come out to play: the molecular biology of dioecious plants". *Annals of Botany* 86.2 (2000): 211-221.

29. Goring DR and Walker JC. "Self-rejection-a new kinase connection". *Science* 303.5663 (2004): 1474-1475.

30. Jaccard P. "Nouvelles researches sur la distribution floral". *Bulletin de la Société Vaudoise des Sciences Naturelles* 44.163 (1908): 223-270.

31. Mantel N. "The detection of disease clustering and a generalized regression approach". *Cancer Research* 27.2 (1967): 209-220.

32. Nei M., *et al*. "Accuracy of estimated phylogenetic trees from molecular data". *Journal of Molecular Evolution* 19.2 (1983): 153-170.

33. Roselino AC., *et al*. "Differences between the quality of strawberries (Fragaria × Ananassa) pollinated by the stingless bees Scaptotrigona aff. depilis and Nannotrigona testaceicornis". *Genetics and Molecular Research* 8.2 (2009): 539-545.

Volume 2 Issue 9 September 2018