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Morphological Variability Assessment of Worldwide Germplasm of Pharmaceutically Important Plant *Nigella Sativa* L.

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ABSTRACT

N. sativa is an important industrial crop globally but neglected crop in Pakistan, hence imported. It has been used for revitalizing body system in almost all ancient civilizations. In the present investigation worldwide accessions of *N. sativa* were cultivated for three years to select the most diverse accessions for cultivation and future breeding purpose in Pakistan. More diverse and acclimatized accessions with enhanced morphological traits were observed. Seed length, seed width, plant height, number of flowers and number of follicles showed maximum considerable variation in three years. Morphological variations observed and collected for all three years were not consistent among all accessions, due to some environmental fluctuations while some of the accessions like ACC 20662, ACC W626529, ACC 20878, ACC 21545, ACC 21428and ACC 20990 showed consistency at some extent among results of different morphological markers (number of flowers, number of stamens, number of follicles, follicle length, follicle width and seed per follicle). 50% accessions germinated in 26-35 days consecutively three years and showed optimum growth. The maximum height was noticed as 108cm in two accessions ACC 20878 and ACC 20780. The maximum frequency of flower initiation days was noted 101-110 days. The maximum frequency distribution for number of branches was noted in frequency class 11-15 during all three years. The maximum frequency of flower initiation days was noted 101-110 days for 2015-16 and 2017-18. The data recorded in 2017-18 depicted the maximum number of flowers as 15 (ACC 20780 and ACC 20878). The rate of follicle production per plant was 10 among accessions (ACC W626529, ACC 20545 and ACC 21295). The largest frequency class for stamens number was 21-25 stamens, whereas the plants with frequency class 31-35 stamens were least frequent. The flower color varied in the recombinant plants (RC) as Purple White whereas in the parents it was White (Jordan) and Bluish white (Ukraine). In general the accessions belonging to Gujranwala, Jordan, Lahore, Haripur, Attock and Sargodha were found morphologically more significant and diverse.

Keywords: Accesions; Cluster; Germplasm; Morphology; Population.

INTRODUCTION

Human beings in all ancient civilizations have been dependent upon herbs for disease treatment and invigorating body systems. An evidence of herbs based treatment of diseases for revitalizing body system in almost all ancient civilization has been observed. [1,2] Now a day, medicinal plants are contemplated as valuable drug source to minimize the perilous effects of synthetic medicines. [3] More than 75% of the global populace is mainly dependent upon plants and their parts to cure hazardous diseases. Approximately 30% or more plant species are annually consumed for medicinal purposes. [4] Herbal formulations being cheaper and non-hazardous,

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have gained the core significance to improve health and international trade. In developing countries the pharmaceutically important plants are playing crucial part in the healthcare schemes of major extents of world's population. [5]

Nigella sativa (Black Seed) from Ranunculaceae is a significant annual herbal plant, indigenous to South and Southwest Asia. [6] The cultivation period ranges from November to April. The preliminary propagating substance of this therapeutically important herb is seed which take 25-30 days to germinate under ideal physical conditions. [7] The archeological evidences narrate the cultivation and utilization of *N. sativa* seed and oil in the ancient civilizations found all around the Mediterranean Sea. [8] The reports regarding cultivation and production potential of *N. sativa* have been documented in Pakistan but very low in production. [6] India is the biggest producer with 25000 matric tones per annum followed by Syria, Turkey, Iran and China. [9] Only N. sativa, N. damascene and N. arvensis are of interest in Jordan; N. sativa is the only species planted by farmers. There is no accurate data about planted area, but the annual production for the year 2005/2006 was 3-5 tons. [10]

The Morphological and biochemical characteristics were explored for the analysis of genetic diversity among indigenous landraces of *Brassica napus* through multivariate techniques. [11] Genetic diversity of *B. napus* landraces was assessed by collecting germplasm of 70 accessions from all over Pakistan. Overall 25 morphological and biochemical markers were analyzed for year 2012 and 2013. The results indicated specific genotypic variability among collected accessions. The maximum variability was perceived in plant height and maturity marker. In addition to these two certain other characters like flower initiation, completion, inflorescence detailed characteristics were also noted. Moreover the biochemical markers were different phytochemical tests. Analysis of results was done through principal component analysis and it divided the whole germplasm into 5 principal components on 2012, whereas the distribution leads to nine components in 2013. Considering two year data, some of the genotypes were found very auspicious with huge genetic diversity and thus recommended for future cultivation.

Various landraces of black cumin (*N. sativa*) in Iran were explored by using morphological parameters, traits related to yield, essential oil and oil production. [12] Traditionally, it has been used as spice and medicine but today an important component of modern pharmaceutical industry, thus highly explored. It has been cultivated in Iran under different geographical locations and climate. The study was planned to explore various landraces for variation in morphology, growth and agricultural yield associated characteristics. *Nigella* cultivation requires less precipitation with warm climate.

N. sativa was used as an experimental material to investigate genetic variation mechanism through multivariate analysis. [13] They planted 32 germplasm accessions of *N. sativa* L. under field conditions for consecutive 3 years 2009-2011. The results were analyzed through principal component analysis and established 6, 3 and 5 PCs during the year 2009, 2010 and 2011 consecutively. The variation among the crops was noted as 87.85%, 79.75% and 79.42% of germplasm, sequentially. The variation detected among the components contributed towards genetic resources conservation. This variation may employ the suitable gene pool in crop enhancement for specific plant traits. Genetic divergence revealed by un-weighed pair group method of arithmetic means (UPGMA). The analysis divided then accessions into eight clusters depending upon three years of field assessment. The Multivariate analysis divulged the accessions belonging to different origins were grouped together indicating that genetic variation was independent of origin. The results also predicted that specific clusters germplasm distribution could be used for better exploration of economic peculiarity and can be a good source towards genetic variation and genetic recombination.

Materials and Methods

Plant Material Collection

75 accessions of *N. sativa* from diverse regions of the world (Pakistan, Jordan, US Idaho and Ukraine) were used in present study (Table 3.1). These areas are considered to be its center of diversity. Among all seed of 70 accessions were collected from Germplasm bank, Bio-resource Conservation Institute, National Agriculture Research Centre (NARC) Islamabad. International accessions were collected from Gene bank United States Department of Agriculture, Germplasm Bank, USDA, Washington DC.

Soil Preparation

Seed of all accessions were planted according to Randomized Complete Block Design (RCBD) in November, 2015 at Botanic garden Lahore College for Women University Lahore, Pakistan located (31.45° N and 74.39° E). Recommended cultural practices and inputs were applied uniformly to all entries during the whole growing season to minimize the field environmental variations. Loamy soil was mixed with farm yard manure and prepared within pH range 5-8. The soil watering was done on daily basis to maintain soil moisture level at 60% (Ghafoor and Ahmad, 2003).

Germplasm Cultivation

The germplasm consisted of 7500 seeds (100 each accession) were planted at Botanic Garden of Lahore College for Women University Lahore. Row distance was kept at 20-30cm. Weeds were controlled manually. The temperature for seed germination was maintained at 20-25ºC. Field conditions were maintained accordingly. [14] Germplasm was planted in November 2015 to April 2016 (P1). The same experiment was repeated for next two years 2016-17 (F1) and 2017-18 (F2) consecutively.

Morphological Markers Selection

Various economically important morphological markers were selected on the basis of available literature[13] The data was recorded randomly selecting ten plants per accession on regular basis for consecutive three years 2015-16, 2016-17 and 2017-18.

Morphological markers under study were; seed length calculated by selecting ten seed per accession and calculated their average value. Seed width was calculated by selecting ten seed per accession and calculated their average value. The total days to germination were noted from right after sowing to 50% germination. The height of plant was noted from ground level to the upper most branches at fruiting stage. Total numbers of branches on each selected accession were counted and calculated their average. The numbers of days from date of sowing till formation of first bud were counted for each accession. Total flowers produced in each accession were counted and calculated their average. The variation in flower/petal color was observed in three years. The petals produced in each accession were counted and noted down their average. Total number of stamens produced in each accession was counted and calculated their average. Total number of follicles produced in each accession was counted and calculated their average. The length of each follicle produced per accession was measured and calculated the average. Width of each follicle produced per plant was measured and calculated the average. Total numbers of seed produced per follicle were counted and calculated their average.

Data Analysis

Data analysis was done through descriptive statistics, Correlation and multivariate analysis (cluster analysis and principal component analysis) using software; SPSS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0 and XLSTAT software. [15,16]

Results

Descriptive analysis of Morphological Markers

To explore the morphological diversity among *N. sativa* germplasm, seventy five accessions were morpho-

metrically evaluated. Seed of all accessions were grown for three years (2015-18) in Botanical Garden LCWU Lahore. Fourteen morphological markers like seed size (length/width), days to germination, plant height, number of branches, days to flower initiation, number of flowers, petal color, number of petals, number of stamens, number of follicles, follicle size (length/ width), number of tentacles and seed per follicle were recorded. Results indicated that accessions differed significantly for all the markers (Table 1). Morphology of accessions revealed presence of a considerable phenotypic diversity that may be utilized for the development of high-yielding cultivars. All morphological markers showed variation among accessions during three consecutive years. Data was recorded in the form of frequency distribution tables following different trait categories.

Table 1: Basic descriptive analysis of quantitative characters of *N. sativa* during 2015-16

Trait	Mean	Maximum	Minimum	SD	Variance
SL	0.40	0.60	0.20	0.03	0.001
SW	0.17	0.25	0.10	4.36	19.03
GD	37.50	55.00	20.00	16.26	264.5
PH (cm)	75.00	120.0	30.0	4.37	19.17
NB	16.50	25.00	8.00	6.28	39.51
DFI	102.50	130.0	75.00	2.84	8.06
NF	12.00	20.00	4.00	0.48	1.29
SN	25.00	35.00	15.00	2.13	4.54
FN	9.50	15.00	4.00	0.28	2.56
FL (cm)	3.00	4.00	2.00	0.23	1.65
FW (cm)	2.00	2.50	1.50	0.50	0.25
TN	5.00	6.00	4.00	0.81	0.86
SF	77.50	95.0	60.00	13.08	171.25

Table 2: Basic descriptive analysis of quantitative characters of *N. sativa* during 2016-17

Trait	Mean	Maximum	Minimum	SD	Variance
SL	0.42	0.60	0.25	0.05	0.003
SW	0.20	0.25	0.15	0.25	0.83
GD	41.5	58.00	25.00	14.65	214.75
PH (cm)	73.00	114.0	32.00	27.42	755.73
NB	14.50	22.00	7.00	7.95	63.33
DFI	106.5	132.0	81.00	40.14	1611.23
NF	12.00	18.00	6.00	5.13	26.39
SN	21.50	31.00	12.00	0.80	0.64
FN	8.00	11.00	5.00	3.72	8.87
FL (cm)	3.25	4.00	2.50	1.34	3.26

Trait	Mean	Maximum	Minimum	SD	Variance
FW (cm)	2.00	2.50	1.50	0.80	2.65
TN	4.50	5.00	4.00	0.72	2.52
SF	63.50	82.00	45.00	13.06	170.73

Key: Abbreviations against morphological markers: Seed Length (SL), Seed Width (SW), Germination Days (GD), Plant Height (PH), Number of Branches (NB), Days to flower Initiation (DFI), Number of Flowers (NF), Stamens Number (SN), Follicle Number (FN), Follicle Length (FL), Follicle Width (FW), Tentacles Number (TN), Seeds per Follicle (SF)

Table 3: Basic descriptive analysis of quantitative characters of *N. sativa* during 2017-18

Trait	Mean	Maximum	Minimum	SD	Variance
SL	0.35	0.45	0.25	0.06	0.005
SW	0.20	0.25	0.15	0.33	0.11
GD	46.00	60.00	32.00	6.10	37.27
PH (cm)	77.00	108.0	46.00	15.76	248.63
NB	11.50	18.00	5.00	13.02	39.14
DFI	111.0	137.0	85.00	14.42	19.53
NF	10.00	15.00	5.00	4.19	18.80
SN	20.00	28.00	12.00	4.17	13.28
FN	7.50	10.00	5.00	2.02	9.46
FL (cm)	3.00	3.50	2.50	1.08	0.39
FW (cm)	2.00	2.50	1.50	0.06	0.11
TN	4.50	5.00	4.00	0.03	0.20
SF	61.50	80.00	43.00	3.07	146.9

Key: Abbreviations against morphological markers: Seed Length (SL), Seed Width (SW), Germination Days (GD), Plant Height (PH), Number of Branches (NB), Days to flower Initiation (DFI), Number of Flowers (NF), Stamens Number (SN), Follicle Number (FN), Follicle Length (FL), Follicle Width (FW), Tentacles Number (TN), Seeds per Follicle (SF)

Seed Length

Seed size is directly proportional to seed contents and increase in seed contents would increase the plant productivity. Seed length showed maximum considerable variation in three years. Seed length ranged from 0.2-0.5cm with mean value of 0.35 cm in 2015-16 (Table 1). The standard deviation was recorded 0.03, whereas the marker showed variance value 0.001 in 2015-16. Seed length was divided into 3 frequency classes (0.2-0.3, 0.31-0.4 and 0.41-0.5) (Table 4). Maximum seed length (0.6cm) was observed in two accessions ACC 20631 and ACC 20766. In 2016-17 maximum seed length was 0.6cm shown by three accessions ACC 20780, ACC 20781 and ACC 20985 with standard deviation 0.05 and 0.003

variance. Seed length of most of the accessions was found in the range of 0.41-0.5cm for two years 2015-16 and 2016-17 (Table 2), whereas in the third year seed length of maximum accessions was in the range of 0.31-0.4cm (46.6%). The highest value for seed length in 2017-18 was 0.45cm (Table 3) recorded in five accessions ACC 20567, ACC 20576, ACC 20742, ACC 20904 and ACC 20976 with standard deviation 0.06 and variance was 0.005 (Table 4).

Seed Width

Seed width is also related to the seed contents. Seed width ranged from 0.1 to 0.25cm, distributed among three frequency classes (0.1-0.15, 0.16-0.2 and 0.21-0.25). The largest frequency class (almost 50%) of seed width in all three years had range 0.16-0.2cm. In 2015-16, maximum seed width was 0.25cm revealed by 15 accessions with 4.36 standard deviation and variance was 19.03 (Table 1). In 2016-17 it was noticed that maximum seed width was 0.25 presented by 15 accessions, while their standard deviation and variance were 0.25 and 0.83 respectively. In 2017-18, the maximum seed width was recorded as 0.25cm expressed in 12 accessions. Their standard deviation was recorded as 0.33, whereas the value of variance was 0.11(Table 4).

Days to Germination

The variation in days to germination is related to seed health and viability. The marker was categorized in four frequency classes (0-25, 26-35, 36-45 and 46-60) (Table 1). The maximum number of days to germination in 2015-16 was 55 (revealed by one accession ACC 22134) with standard deviation 16.26 and variance was 264. In 2016-17, the maximum days to germination were 58 (ACC 20545, ACC 20781, ACC 20985 and ACC 20904 (Table 2). Their standard deviation and variance were recorded 14.65 and 214.75 43 respectively. Approximately 50% accessions germinated in 26-35 days consecutively three years, thus it could be identified as optimal duration for seed germination. In 2017-18 the maximum number of days to germination were 60 (ACC 20561, ACC 20981 and ACC 20781) with standard deviation 6.10 and variance was 37.27(Table 3).

Plant Height

High level of variability was detected in plant height among tested germplasm. The marker was categorized in four frequency classes (0-40, 41-60, 61-90 and 91-120) (Table 1). In the first year trial (2015-16), the maximum plant height (120cm) was exhibited by one accession ACC W626529 (Jordan) with standard deviation 4.37 and variance was 19.17. In the second year trial (2016-17) the maximum plant height was 114cm noted in four accessions ACC 20561, ACC 20878, ACC 20985 and ACC 25910 (Table 2). Their standard deviation and variance values were 27.42 and 755.73 respectively. Overall low plant heights were noticed lesser in 2017-18 field trials. The maximum height was noticed as 108cm in two accessions ACC 20878 and ACC 20780, while standard deviation and variance were 15.76 and 248.63 (Table 3). The maximum accessions were found in the frequency class ranging 61-90cm height for 2015-16 and 2017-18 field trial however, the maximum frequency of plant height was observed in between 41-60cm during the year 2016-17 (Table 4).

Number of Branches

The accessions showed distinct variation in number of branches for three years field trials. The marker was distributed among four frequency classes (1-10, 11-15, 16-20 and 21-25) (Table 4). During first field trial the maximum numbers of branches were 25 (ACC W626529), standard deviation was 6.28 whereas the variance was 39.51. During 2016-17 the maximum numbers of branches were 22 (ACC 20561 and ACC 20878) with standard deviation 7.95, while the variance was recorded as 63.33. In the next year (2017-18) (Table 3) the maximum number of branches were 18 (ACC 20878, ACC 20780) and the values for standard deviation and variance were 13.02 and 39.14 respectively. The maximum frequency distribution for number of branches was noted in frequency class 11-15 during all three years (Table 4)

Days to Flower Initiation

N. sativa germplasm depicted highly variable days to flower initiation in three years (Table 4).The marker ranged from 1-140 days consisting of four frequency distribution classes (1-100, 101-110, 111-120 and 121-140) (Table 3). In 2015-16, maximum number of days to flower initiation were130 (ACC 20881 and ACC 21208), where their standard deviation and variance values were 2.84 and 8.06 respectively. The next year (2016-17), maximum days to flower initiation increased to132 (ACC 21262, ACC 20781, ACC 21295 and ACC 21428) with standard deviation 40.14 and variance was 1611.23 (Table 4). In 2017-18, maximum days to flower initiation showed further increase (137). Two accessions ACC 20545 and ACC 20990 were recorded within this category. This year standard deviation and variance were 14.42 and 19.53 respectively. The maximum frequency of flower initiation days was noted 101-110 days for 2015-16 and 2017-18, whereas maximum frequency of flower initiation in 2016-17 was 111-120 days distribution class.

Number of Flowers

The marker was distributed into four frequency classes (1-5, 6-10, 11-15 and 16-20). The maximum number of flowers in 2015-16 were 20 (ACC W626529 and ACC 20662) and their standard deviation and variance values were 0.48 and 1.29 respectively (Table 1). In the next year (2016-17) maximum value for flower number was 18 (ACC 20561 and ACC 20878), though standard deviation and variance were 5.13 and 26.39 (Table 2). The data recorded in 2017-18 depicted the maximum number of flowers as 15 (ACC 20780 and ACC 20878) with standard deviation 4.19 while the variance was 18.80 (Table 3). The number of petals varied from 5-6 in three years.

Stamens Number

An enormous variation was recorded in the number of stamens. The marker was divided in four frequency distribution classes (0-20, 21-25, 26-30 and 31-35). In the first field trial (2015-16), maximum stamens was 35(ACC 21208 and ACC 21544) (Table 1). Their standard deviation and variance were 2.13 and 4.14 respectively. In the second field experiment (2016-17) maximum stamen was recorded 31(shown by three accessions ACC 21662, ACC 21878 and ACC 21545), while standard deviation and variance values were 0.80 and 2.65 (Table 2).. Next field experiment (2017-18) showed maximum 28 stamens (ACC 20699, ACC 20990, ACC 21090 and ACC 21428), however the values for standard deviation and variance were 4.17 and 13.28 respectively (Table 3).. The largest frequency class for all three years was 21-25 stamens, whereas the plants with frequency class 31-35 stamens were least frequent (Table 4).

Number of Follicles

The number of follicles is considered as one of the vital traits related to plant productivity. It was also observed as significant variable marker among accessions. The marker was divided into three frequency classes (1-5, 6-10 and 11-15) (Table 4). In the first experimental trial maximum 15 follicles per plant (ACC 21208, ACC 21662, ACC 20878 and ACC 21545) were observed with standard deviation 0.28 whereas variance was 2.56 (Table 1). Decreasing trend in number of follicles per plant was noticed in the next year. Maximum number of follicles produced per plant were 11(ACC21099 and ACC 20878) with standard deviation 3.72 while variance was 8.87 (Table 2). The next year (2017-18) rate of follicle production per plant was 10 (ACC W626529, ACC 20545 and ACC 21295), standard deviation and variance were 2.02 and 9.46 (Table 3).

Follicle Length

The size of follicle is a significant character, as there is an increase in size there would be more number of seed produced. The marker was distributed into three frequency classes (1-2.5, 2.6-3 and 3.1-4). In 2015-16, the maximum follicle length was recorded as 4cm (among 4 accessions ACC 20878, ACC 20662, ACC21090 and ACC 21355), however their standard deviation and variance values were 0.23 and 1.65 (Table 1). The next field trial pretended that maximum length of follicle was again 4cm (among two accessions ACC 21090 and ACC 21432) but standard

deviation and variance values were 1.34 and 3.26. The last field trail depicted the maximum follicle length as 3.5cm (among three accessions ACC W626529, ACC 21545 and ACC 20662) with standard deviation 1.08 and variance was 0.39 (Table 2). The maximum frequency of plants with follicle length were found in the frequency class 1-2.5cm for the 3 years, but some of the accessions possessed comparatively greater length frequency in the range of 3.1-4 (Table 4).

Follicle Width

Follicle length is another distinguishing character related to plant productivity, increase in width would increase the number of seed production. The marker was distributed among three frequency classes (1-1.5, 1.6-2 and 2.1-2.5) (Table 4). The maximum width of follicle for all the three years remained same (2.5cm) but their standard deviation and variance values varied depending upon the frequency of individuals. In 2015-2016 three accessions revealed the maximum follicle width (ACC 20662, ACC 21416 and ACC 21428), while standard deviation and variance values were 0.50 and 0.25 (Table 1). The next year (2016-17) maximum follicle width was observed in four accessions (ACC 20878, ACC 20990, ACC 21090 and ACC 21432). Their standard deviation and variance values were 0.80 and 0.65 respectively (Table 3). Last field trial showed the presence of two accessions with maximum width (ACC 20662 and ACC W626529) while their values for standard deviation and variance were 0.06 and 0.11 (Table 4).

Table 4: Frequency distribution of different morphological markers

Seed Length (cm)						
Frequency Class	**2015-16**		**2016-17**		**2017-18**	
	Frequency	**% age**	**Frequency**	**% age**	**Frequency**	**% age**
0.2-0.3	17	22.6	20	26.6	15	20
0.31-0.4	25	33.3	25	33.3	35	46.6
0.41-0.5	33	44	30	40	25	33
Seed Width (cm)						
0.1-0.15	17	22.6	22	29.3	18	24
0.16-0.2	32	42.6	37	49.3	37	49.3
0.21-0.25	26	34.6	16	21.3	20	26.6
Days to Germination						
0-25	12	16	15	2	17	22.6
26-35	45	80	50	66.6	45	80
36-45	10	13.3	8	10.6	12	16
46-60	8	10.6	2	2.6	1	1.3
Plant Height (cm)						
0-40	9	12	16	21.3	20	26.6
41-60	28	37	33	44	19	25.3
61-90	32	42.6	20	26.6	27	36
91-120	6	8	6	8	9	12
Number of Branches						
1-10	25	25	22	23.9	20	26.6
11-15	34	34	33	35.8	33	44
16-20	28	28	25	27.1	16	21.3
21-25	13	13	7	7.6	6	8
Days to Flower Initiation						
1-100	10	13.3	9	12	11	14.6

Seed Length (cm)						
Frequency Class	**2015-16**		**2016-17**		**2017-18**	
	Frequency	**% age**	**Frequency**	**% age**	**Frequency**	**% age**
101-110	30	40	28	37.3	34	45.3
111-120	27	37	35	46.6	22	29.3
121-140	8	10.6	3	4	8	10.6
Number of Flowers						
1-5	8	10.6	10	13.3	11	17.3
6-10	23	30.6	28	37.3	28	37.3
11-15	34	45.3	29	38.6	30	37.3
16-20	10	13.3	8	10.6	6	8
Petal Color						
White	48	46	48	46	48	46
Blue	1	0.01	1	0.01	1	0.01
Pale White	26	34.6	26	34.6	26	34.6
Bluish White	0	0	0	0	1 (RC.)	-
Number of Stamens						
0-20	20	26.6	22	23.9	16	21.3
21-25	32	42.6	46	50	34	45.3
26-30	15	20	20	21.7	17	22.6
31-35	8	10	4	4.3	8	10.6
Follicle Number						
1-5	27	36	24.6	32	36	48
6-10	32	42.3	37	49	31	41.3
11-15	16	21	14	18	8	10.6
Follicle Length (cm)						
1-2.5	35	46.6	37	49.3	38	50.6
2.6-3	27	36	33	44	31	41.3
3.1-4	13	17.3	5	6.6	6	8
Follicle Width (cm)						
1-1.5	35	46.6	37	49.3	34	45.3
1.6-2	30	40	26	34.6	28	37.3
2.1-2.5	10	13.3	12	16	13	17.3
Number of Tentacles						
1- 4.0	38	41.3	37	40.2	35	46.6
4.1-5.0	40	43.4	40	43.4	35	46.6
5.1-6.0	14	15.2	15	16.3	15	20
Number of Seed per Follicle						
60-70	25	33.3	22	29.3	20	26.6
71-80	28	37.3	35	46.6	32	42.6
81-90	16	21.3	11	14.6	12	16
91-95	9	12	7	9.3	11	14.6

Number of Tentacles

The number of tentacles was also variable among the accessions. The marker was distributed among three frequency classes (1-4, 4.1-5 and 5.1-6). This trait was divided into 3 categories like 4, 5 and 6 (Table 4). In 2015-16, the maximum number of follicle tentacles was six

observed in 46 accessions, while their standard deviation and variance values were 0.81 and 0.86 (Table 1). In 2016-17 the maximum tentacles were six present in 39 accessions, whereas their standard deviation and variance values were 0.72 and 0.52. In 2017-18 the maximum tentacles were again six in 40 accessions with standard deviation 0.03 and variance was 0.20. The accessions found in frequency class 4.1-5 were most frequent among all the accessions of 3 years 2015-16, 2016-17 and 20 17-18.

Seed per Follicle

An enormous variation was observed in the number of seed per follicle. Seed productivity is directly related to plant yield. This marker was further categorized in 4 frequency classes like 60-70, 71-80, 81-90 and 91-95 seed per follicle. The maximum number of seed produced per follicle in 2015-16 were 95 (ACC 20662 and ACC 21428) with standard deviation 13.08 and varianc3 171.25. In 2016-17 the maximum seed production per follicle was 82 (ACC 20878 and ACC 20990), however standard deviation and variance were 13.06 and 170.73 (Table 2). Seed production in 2018 was maximum 80 seed per follicle (ACC 20662 and ACC W626529) while standard deviation and variance recorded were 3.07 and 146.9. The accessions having 83-85 seeds per follicle were the most frequent among all accessions in 3 cultivating years (Table 4).

Petal Color

Like all the quantitative morphological markers it was also distributed into four frequency classes (White, blue, pale white and bluish white). In literature white and pale white color of flowers is reported. Flower/petal color also varied among accessions producing three types of flower colors like white, blue and pale white in 2015-16 and 2016-17. Whereas, an additional type of flower color (bluish white) was observed in recombinant of W626529 (Jordan) and W626530 (Ukraine) selected from the two centers of *N. sativa* diversity (Table 4). The flowers with white petal color were most frequent among all the accessions in 2015-18.

One hundred and fifty plants belonging to W626529 (Jordan) and W626530 (Ukraine) each were cross pollinated in 2016-2017 that resulted in the production of fifty cross pollinated plants with new flower color (bluish white) was observed. Later on the seeds produced from these plants were again sown in 2017-18 to check seed viability and morphological variations occurred among all morphological markers. Both qualitative and quantitative characters showed variation in the recombinant plants. There was slight increase in plant height, number of branches, number of flowers and number of follicles in the recombinant plant. Likewise the flower color also varied in the recombinant plants (RC) as Purple White whereas in the parents it was White (Jordan) and Bluish white (Ukraine). The results from morphological marker analysis indicated that variations were not consistent for three years among all the accessions but a few markers like number of flowers, number of stamens, number of follicles, follicle length, follicle width and seed per follicle exhibited the maximum diversity among some of the accessions for three year like ACC 20662, ACC W626529, ACC 20878, ACC 21545, ACC 21428 and ACC 20990.

Correlation and Principle Component Analysis of quantitative morphological markers

On the basis of morphological markers, correlation among seventy five accessions of *N. sativa* was calculated for three years data separately. The average values of 14 morphological markers were analyzed for simple correlation coefficient. The morphological markers, seed size, germination days, plant height, number of branches, number of flowers and number of follicles etc. were considered for this analysis. The correlation coefficient among markers was calculated using statistical software MEGA X and SPSS 20. Positive and negative correlation was observed among variable markers. The range of correlation among the morphological markers varied from 0.01- 0.83 in 2015-16, 0.02-0.90 in 2016-17 and 0.02-0.80 in 2017-18 (Table 7). The correlation calculated for 2015-16 indicated that seed length has positive correlation with

days to germination (r^2= 0.24) number of branches (r^2= 0.06), number of flowers (r^2= 0.17), number of petals (r^2= 0.06), number of stamens (r^2= 0.02), number of tentacles (r^2= 0.02) and seed per follicle (r^2= 0.06), whereas negative correlation with plant height (r^2= - 0.65) , flower initiation days (r^2= - .07) number of follicles (r^2= - 0.07), follicle length (r^2= - 0.03) and follicle width (- 0.05). The maximum positive correlation value (r^2= 0.83) was observed between plant height and number of branches followed by correlation between flower initiation days and stamen numbers (r^2= 0.62). It was also observed that increase in seed length negatively correlated the follicle length and width. Likewise days to germination exhibited positive correlation with plant height, number of branches, number of flowers, number of petals, follicle length and follicle width.

Table 5: Correlation of quantitative morphological markers of *N. sativa* L. (2015-16)

Morphological Markers	SL	SW	GD	PH	BR	FID	FN	PN	SN	FN	FL	FW	TN	SF
SL	1.000													
SW	.304	1.000												
GD	.244	-.079	1.000											
PH	-.065	-.200	.003	1.000										
BR	.061	-.267	.040	.835	1.000									
FID	-.077	.298	-.221	.222	.156	1.000								
FN	.173	.480	.095	-.127	-.228	.170	1.000							
PN	.063	.128	-.020	-.141	-.081	-.020	-.021	1.000						
SN	.024	.169	-.124	-.039	-.087	.628	.176	.062	1.000					
NF	-.072	-.079	-.042	.135	.087	-.119	.061	-.099	-.032	1.000				
FL	-.036	.031	.034	.142	.136	-.025	-.039	.072	.120	.097	1.000			
FW	-.055	-.119	.031	.314	.208	-.217	-.157	.059	-.009	.038	.348	1.000		
TN	.023	.041	.031	.011	-.016	-.019	.025	.053	.019	-.051	.013	.011	1.000	
SF	.065	.251	.096	-.087	-.095	-.036	-.012	.127	-.067	-.142	.079	-.007	.034	1.000

Key: Abbreviations of Morphological Markers Seed Length (SL), Seed Width (SW), Germination Days (GD), Plant Height (PH), Branches Number (BN), Flower Initiation Days (FID), Flowers Number (FN), Petals Number (PN), Stamens Number (SN), Number of Follicles (NF), Follicle Length (FL), Follicle Width (FW), Tentacles Number (TN), Seeds per Follicle (SF)

In the correlation among morphological markers of 2016-17, it was noticed that most of the markers showed their positive correlation with one another and only few markers were negatively correlated like seed width and seed length (Table 6). Flower initiation days/ petals numbers exhibited the maximum positive correlation (r^2= 0.09), followed by the positive correlation of petal numbers and stamen numbers (r^2= 0.86) and the between flower initiation days and stamen numbers (r^2= 0.82). Days to germination showed positive correlation with all the morphological markers under observation. It was noticed that seed width had negative correlation with some of markers like germination days (r^2= -0.06), stamen number (r^2= - 0.06), follicle length (r^2= - 0.08), follicle width (r^2= - 0.15), tentacles number (r^2= - 0.18) and seed per follicle (r^2= - 0.16) and positive correlation with all other markers. Seed length showed negative correlation with follicle width (- 0.04) and tentacle number (r^2= - 0.07).

Table 6: Correlation of quantitative morphological markers of *N. sativa* L. (2016-17)

Morphological Markers	SL	SW	GD	PH	BR	FID	FN	PN	SN	FN	FL	FW	TN	SF
SL	1.000													
SW	0.123	1.000												
GD	0.101	-0.066	1.000											
PH	0.060	0.088	0.496	1.000										
BR	0.101	0.239	0.132	0.646	1.000									
FID	0.045	0.016	0.726	0.534	0.391	1.000								
FN	0.014	0.102	0.324	0.687	0.793	0.479	1.000							
PN	0.132	0.024	0.746	0.562	0.416	0.903	0.503	1.000						
SN	0.028	-0.062	0.697	0.485	0.336	0.829	0.456	0.867	1.000					
NF	0.119	0.089	0.216	0.598	0.644	0.533	0.704	0.491	0.453	1.000				
FL	0.079	-0.087	0.560	0.439	0.228	0.603	0.437	0.636	0.607	0.442	1.000			
FW	-0.041	-0.158	0.487	0.507	0.202	0.651	0.320	0.611	0.480	0.398	0.567	1.000		
TN	-0.076	-0.186	0.030	0.121	0.110	0.086	0.285	0.114	0.070	0.175	0.395	0.362	1.000	
SF	0.185	-0.163	0.097	0.131	0.034	0.137	0.135	0.165	0.021	0.061	0.221	0.217	0.225	1.000

Key: Abbreviations of Morphological Markers Seed Length (SL), Seed Width (SW), Germination Days (GD), Plant Height (PH), Branches Number (BN), Flower Initiation Days (FID), Flowers Number (FN), Petals Number (PN), Stamens Number (SN), Number of Follicles (NF), Follicle Length (FL), Follicle Width (FW), Tentacles Number (TN), Seeds per Follicle (SF)

In 2017-18 the maximum positive correlation (r^2= 0.08) was observed between numbers of branches and follicle length, followed by correlation between follicle length and petals number (r^2= 0.062). As the number of branches increased the length of follicles increased. Flower initiation days exhibited positive correlation with all the morphological markers like flowers number (r^2= 0.16) petals number (r^2= 0.23), stamens number (r^2= 0.13), follicle number (r^2= 0.14), follicle length (r^2= 0.21), follicle width (r^2= 0.08), tentacle number (r^2= 0.05) and seed per follicle (r^2= 0.24). The maximum negative correlation was found between seed length and tentacles number (r^2= -0.2). Tentacle number had negative correlation with maximum number of morphological markers thus caused negative effect on their growth (Table 7).

Table 7: Correlation of quantitative morphological markers of *N. sativa* L. (2017-18)

Morphological Markers	SL	SW	GD	PH	BR	FID	FN	PN	SN	FN	FL	FW	TN	SF
SL	1.000													
SW	.061	1.000												
GD	.106	.048	1.000											
PH	.152	.062	0.333	1.000										
BR	.080	.034	-.053	0.655	1.000									
FID	.128	.011	0.284	0.251	-.087	1.000								
FN	.064	.101	0.197	0.745	0.681	.169	1.000							
PN	0.032	0.05	0.167	0.231	0.145	0.231	0.12	1.000						

Morphological Markers	SL	SW	GD	PH	BR	FID	FN	PN	SN	FN	FL	FW	TN	SF
SN	.019	-.042	.059	.084	.021	.133	.033	0.08	1.000					
NF	.173	-.062	.019	0.496	0.419	.145	0.499	0.03	.135	1.000				
FL	-.017	.114	0.288	0.229	.802	0.214	0.272	0.67	.120	.181	1.000			
FW	-.062	.157	-0.213	.030	.058	.080	-.077	0.43	.086	.122	0.229	1.000		
TN	-.203	-.074	-.106	-.034	.043	.053	-.022	0.54	.071	-.104	.039	.151	1.000	
SF	.170	.037	.264	.160	.083	.246	.172	0.23	-.037	.025	.099	-.059	-.103	1.000

Key: Abbreviations of Morphological Markers Seed Length (SL), Seed Width (SW), Germination Days (GD), Plant Height (PH), Branches Number (BN), Flower Initiation Days (FID), Flowers Number (FN), Petals Number (PN), Stamens Number (SN), Number of Follicles (NF), Follicle Length (FL), Follicle Width (FW), Tentacles Number (TN), Seeds per Follicle (SF)

Multivariate analysis of *N. sativa* Accessions on the basis of morphological markers

The multivariate analysis of *N. sativa* germplasm was carried out using two corresponding approaches; principal component analysis and cluster analysis.

Distribution of various accessions through Principal Component Analysis (PCA)

Principal component analysis of accessions was done for three years individually. The purpose of principal component analysis was to explain the variance-covariance relationship among variables through their linear combinations. The accessions among all years did not exhibit too many variations. Uniformity in distribution was because of consistency in few characters like number of petals (5-6) and number of tentacles (4-6) remained almost same in 3 years. PCA divided into two components Principal component 1(PC1) Principal component 2 (PC2). The values for PC1 and PC2 varied three years 2015-16, 2016-17 and 2017-18. In 2015-16 the accessions distribution was quite uniform among all the regions. PC1/F1 had a contribution ratio of 15.30%, while PC2/F2 contributed11.10% for distribution of factors. Overall contribution of both factors was 26.40%. PC1 and PC2 axis distributed the accessions based on morphological markers variation. PCA score plot showed the contribution of various morphological markers for the placement of accessions in different regions. Some accessions were placed on the X-axis, both at positive and negative side, while the others were found near to the Y-axis. PCA for the year 2015-16 revealed that there was not a huge difference in the morphological markers of Ukraine, Layyah, Rawalpindi, Jordan, Jhang and Lahore found in same distribution component. Likewise no remarkable morphological variation was noted in the accessions belonging to US Idaho, Sukkar, Khushab, Jordan and Faisalabad (Fig. 1). The distribution pattern narrated that the placement of various accessions was not because of single factor but due to contribution and correlation of various morphological markers.

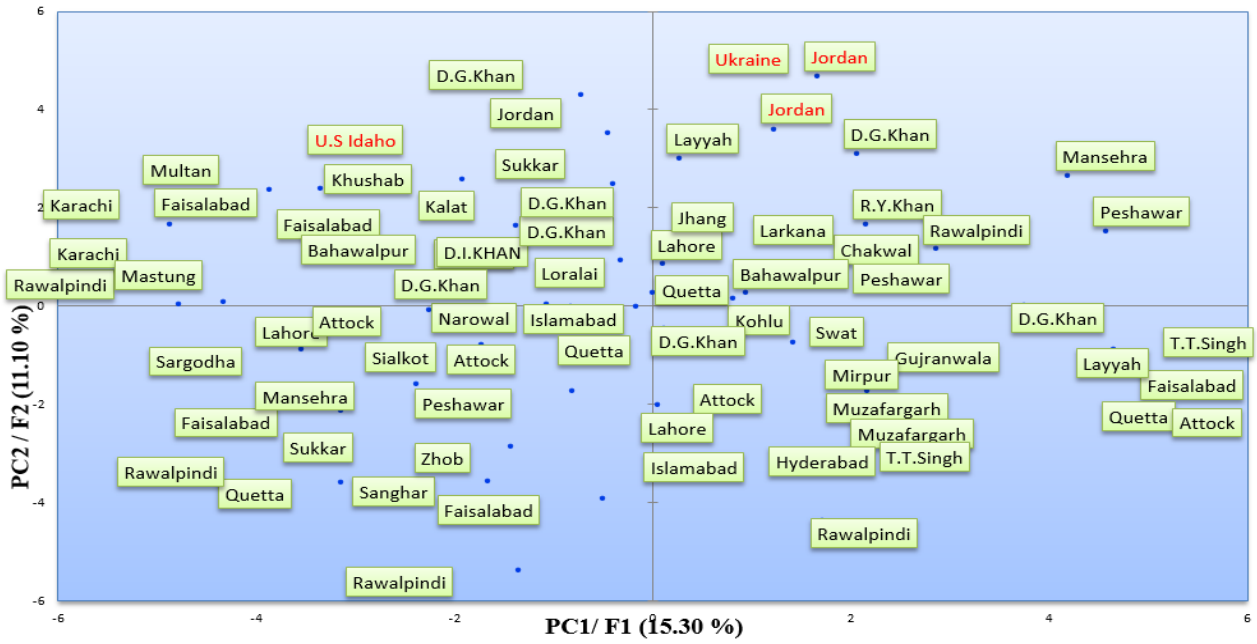

Fig.1: PCA of various accessions in 2015-16 based on morphological markers variation, Score plot for PC1 vs PC2. Percentage values in brackets are contribution values of markers

PCA analysis for the year 2016-17 was different from the previous year. This time PCA placed maximum numbers of accessions in two components, quite near to X-axis, both at positive and negative side. PC 1 contributed 36.24%, whereas PC2 was at 7.98%. Overall contribution of both factors was noted as 44.22%. PCA showed that there was no remarkable difference in the morphological markers of the indigenous and international accessions the accessions belonging to Ukraine, Jordan, Lahore, Chakwal, D.I.Khan and Bahawalpur were placed in close proximity in the same component (Fig. 2). Likewise same distribution position was followed the accessions from Loralai, Larkana, D.G.Khan, T.T.Singh, R.Y.Khan and Quetta.

The distribution pattern in the year 2017-18 was quite identical to the placement in 2015-16 (Fig. 3). Although, origin of accessions was different but the distributed among all the components was quite uniform. Overall principal component contribution was observed as 31.12%. PC1 contributed 21.78%, while that of PC2 was 9.34% . PCA placed the Recombinant plant, Jordan, Ukraine, Layyah, U.S. Idaho, Quetta, Kalat, Khushab, Muzaffargarh, Rawalpindi and Swat accessions in the same components based on their morphological similarities. Likewise the accessions belonging to Chakwal, Multan, Bahawalpur, Mansehra, Attock and Lahore were kept in the same region.

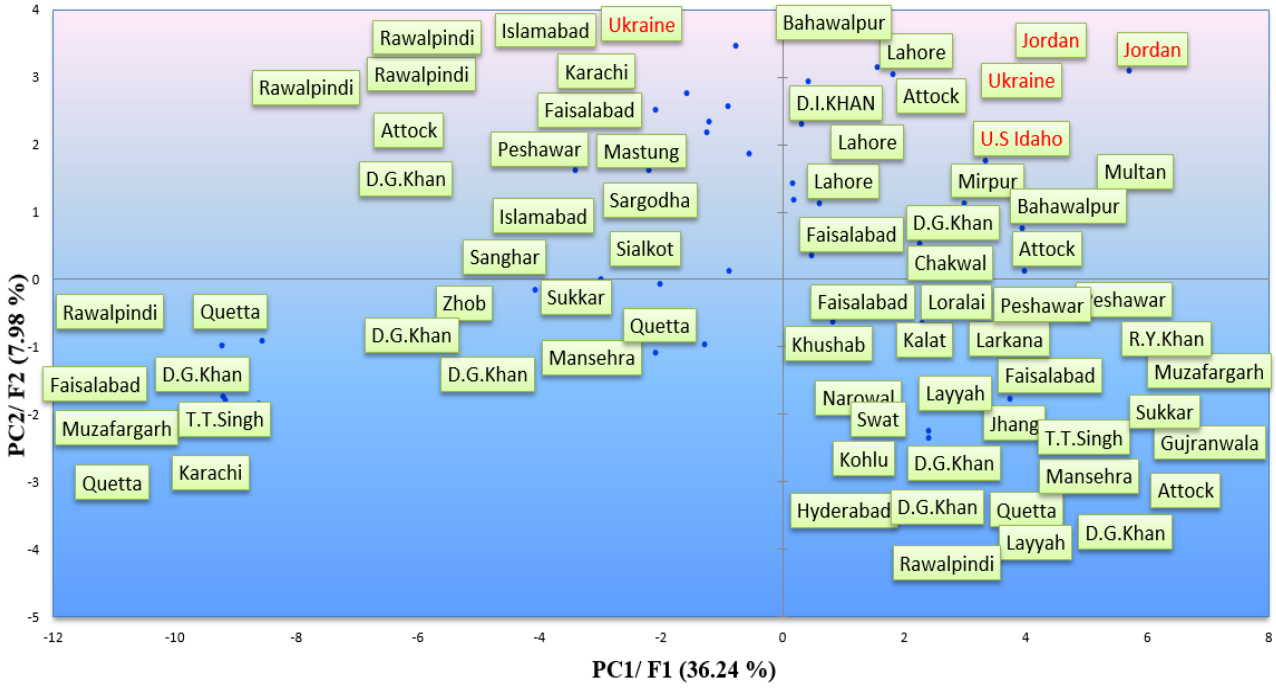

Fig. 2: PCA of various accessions in 2016-17 based on morphological markers variation, Score plot for PC1 vs PC2. Percentage values in brackets are contribution values of markers

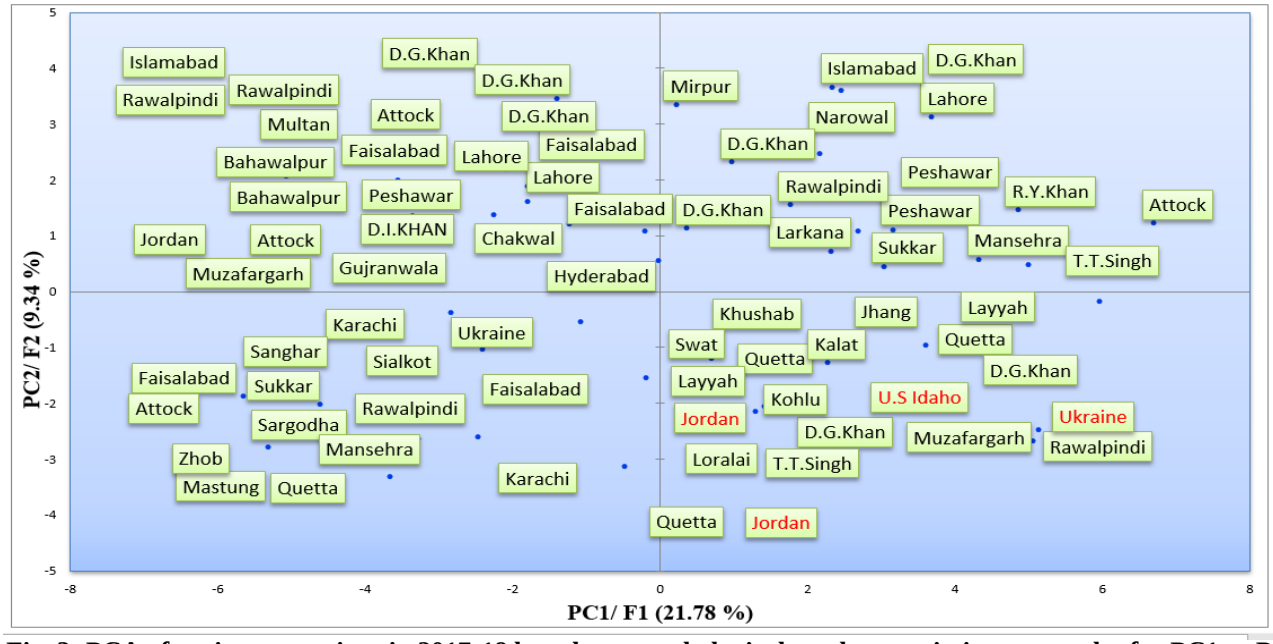

Fig. 3: PCA of various accessions in 2017-18 based on morphological markers variation, score plot for PC1 vs PC2. Percentage values in brackets are contribution values of markers

Distribution of various accessions among clusters through morphological markers

Hierarchical clusters of seventy five accessions of *N. sativa* L. were constructed for consecutive three years i.e. 2015-16 (Cluster I), 2016-17 (Cluster II) and 2017-18 (Cluster III) were constructed on the basis of morphological markers by Ward Linkage method.

In 2015-16 at 100% Ward linkage, all the accessions were divided into two main clusters. The differentiating morphological markers were seed Germination days, plant height, number of branches, flower initiation days, number of flowers and seeds per follicle. Cluster 1 was further divided into two sub-clusters 1A and IB on the basis of differences between number of stamens and number of follicles. 1A was subdivided into two sub sub-clusters 1Ai and 1Aii due to the difference between seeds height and seeds width. Sub sub-cluster 1Ai consisted of 18 accessions including thirteen local accessions ACC25914, ACC25915, ACC21295, ACC21777, ACC21395, ACC21777, ACC20904, ACC21362, ACC21432, ACC22290, ACC21475, ACC20567, ACC21090 and five international accessions like ACCW626529, ACCW626530, ACCW626531, ACCP1506432 and ACCW618059. 1Aii contained only two accessions ACC20609 and ACC20620. Both the accessions belonged to Peshawar. Cluster 1B was further divided into two sub sub-clusters 1Bi and 1Bii based on the difference between follicle height and follicle width. 1Bi possessed 21 accessions ACC21355, ACC21807, ACC20742, ACC21805, ACC20654, ACC20749, ACC21775, ACC20880, ACC21778, ACC20781, ACC20783, ACC20881, ACC25910, ACC22099, ACC25912, ACC25913, ACC21774, ACC20676, ACC20631, ACC20780, ACC20662, whereas only 6 accessions were found in the category 1Bii. Cluster 2 was divided into 2 sub- clusters 2A and 2B based on differences between the numbers of petals and numbers of tentacles. Sub-cluster 2A possessed 13 accessions ACC20545, ACC21724, ACC22083, ACC20786, ACC21208, ACC20561, ACC21776, ACC21017, ACC21762, ACC21428, ACC22147, ACC22115, ACC22134 and remaining eight accessions ACC20976, ACC21967, ACC20592, ACC20679, ACC20990, ACC22301, ACC22053 and ACC21753 were found in sub-cluster 2B. The presence of no distinct pattern of hierarchical distribution for international accessions was observed in cluster 2015-16 (Fig. 4).

In 2016-17 the cluster exhibited sufficient morphological variation. It was divided into two main clusters 1 and 2 on the basis of main differentiating markers like seed germination days, plant height, number of branches, flower initiation days, number of flowers and seeds per follicle. Cluster 1 was further divided into two sub-clusters 1A and 1B based on seed height, seed width, and number of petals variation. Cluster 1A possessed 23 accessions like ACC20699, ACC21416, ACC20985, ACC25911, ACC20576, ACC20585, ACC21724, ACC20878, ACC20545, ACC22083, ACC20766, ACC21208, ACC20561, ACC21776, ACC21206, ACC20561, ACC21766, ACC21017, ACC21762, ACC21428, ACC22147, ACC22115 and ACC22134, whereas cluster 1B contained 9 accessions ACC20976, ACC22301, ACC20592, ACC20990, ACC20879, ACC20874, ACC22053, ACC21753, ACC21967. Cluster 2 was further divided into two sub-clusters 2A and 2B based on number of stamens and numbers of follicles (Fig 5). Based on variation in follicle height and follicle width 2A contained two accessions ACC20609 and ACC20620. Sub-cluster 2B was further sub divided into two sub sub-clusters 2Bi and 2Bii having difference between marker. 2Bi contained eleven local accessions ACC25914, ACC25915, ACC21536, ACC21777, ACC20904, ACC21382, ACC21432, ACC22293, ACC21475, ACC20567, ACC21090 and four international accessions ACCW626531, ACCW626530, ACCW626529 and ACCW610859. Sub sub-cluster 2Bii contained 28 accessions ACC25912, ACC25913, ACC21774, ACC20662, ACC21544, ACC21385, ACC21612,

ACC22155, ACC20742, ACC20877, ACC20749, ACC21295, ACC21805, ACC20654, ACC21275, ACC20631, ACC20780, ACC20896, ACC20881, ACC25910, ACC21382, ACC21355, ACC21807, ACC20880, ACC21778, ACC20781, ACC20783 and ACC22099.

In 2017-18 hierarchical distribution was into two main clusters 1 and 2. Major differentiating morphological markers responsible for this distribution were seed germination days, plant height, and number of branches, flower initiation days, number of flowers and number of seeds per follicle. Cluster 1 further divided into two sub-clusters 1A and 1B based on seed height, seed width, number of petals, number of follicles, follicle height and follicle width. Cluster 1A contained 24 local accessions ACC22099, ACC25913, ACC20783, ACC21607, ACC20654, ACC25910, ACC20631, ACC21544, ACC22147, ACC20742, ACC21778, ACC20699, ACC21774, ACC21805, ACC21295, ACC20783, ACC21775, ACC21355, ACC21395, ACC20904, ACC21362, ACC22290, ACC21090, ACC21777 and five international accessions ACCW618059, ACCW626531, ACCW626529, ACCP1506432, ACCW656530 and Recombinant (RC) was also included in this cluster. Sub-cluster 1B possessed 30 accessions like ACC20620, ACC20661, ACC22063, ACC25911, ACC21047, ACC22301, ACC20501, ACC22115, ACC25914, ACC20990, ACC21762, ACC20592, ACC20662, ACC21250, ACC22003, ACC25912, ACC20609, ACC20545, ACC20745, ACC21428, ACC25915, ACC20706, ACC21416, ACC20690, ACC21724, ACC20985, ACC21229, ACC20768, ACC20874 and ACC576. Remaining eight accessions ACC21612, ACC21967, ACC20585, ACC21535, ACC21382, ACC22155, ACC20976, ACC20877 were grouped in cluster 3 (Fig. 6)

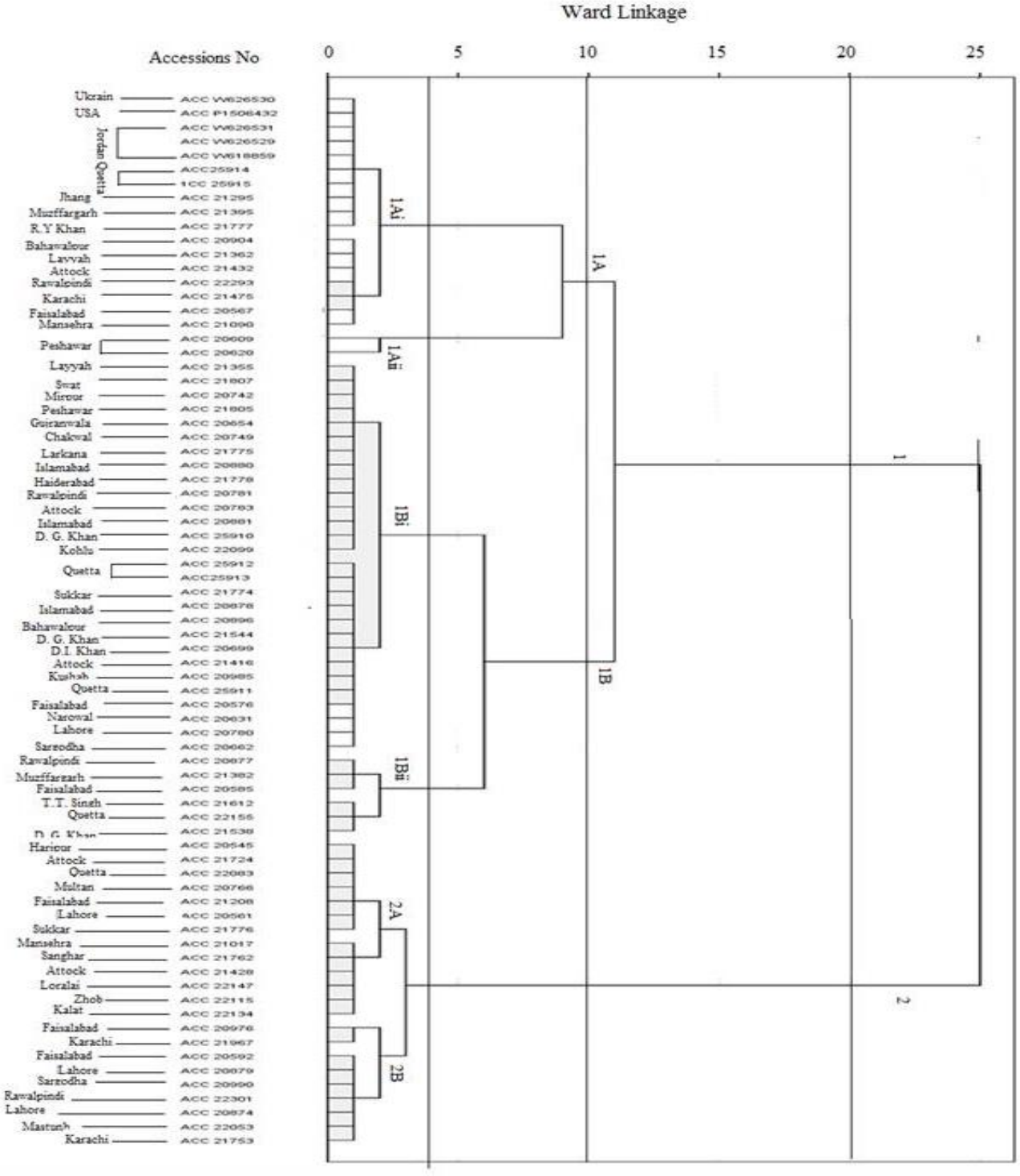

Fig. 4: Distribution of various accessions among clusters through Ward linkage for 2015-16

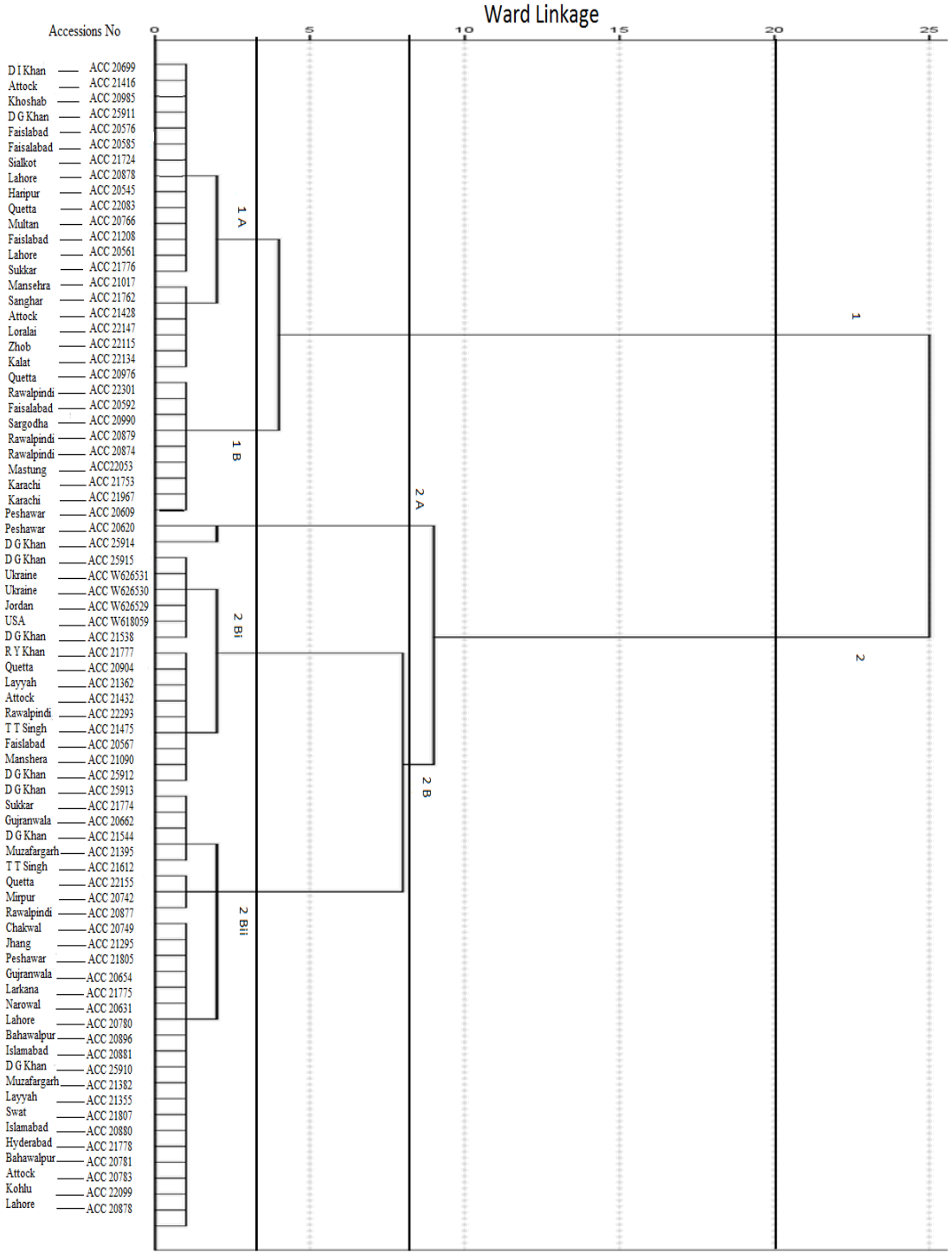

Fig. 5: Distribution of various accessions among clusters through Ward linkage for the year 2016-17

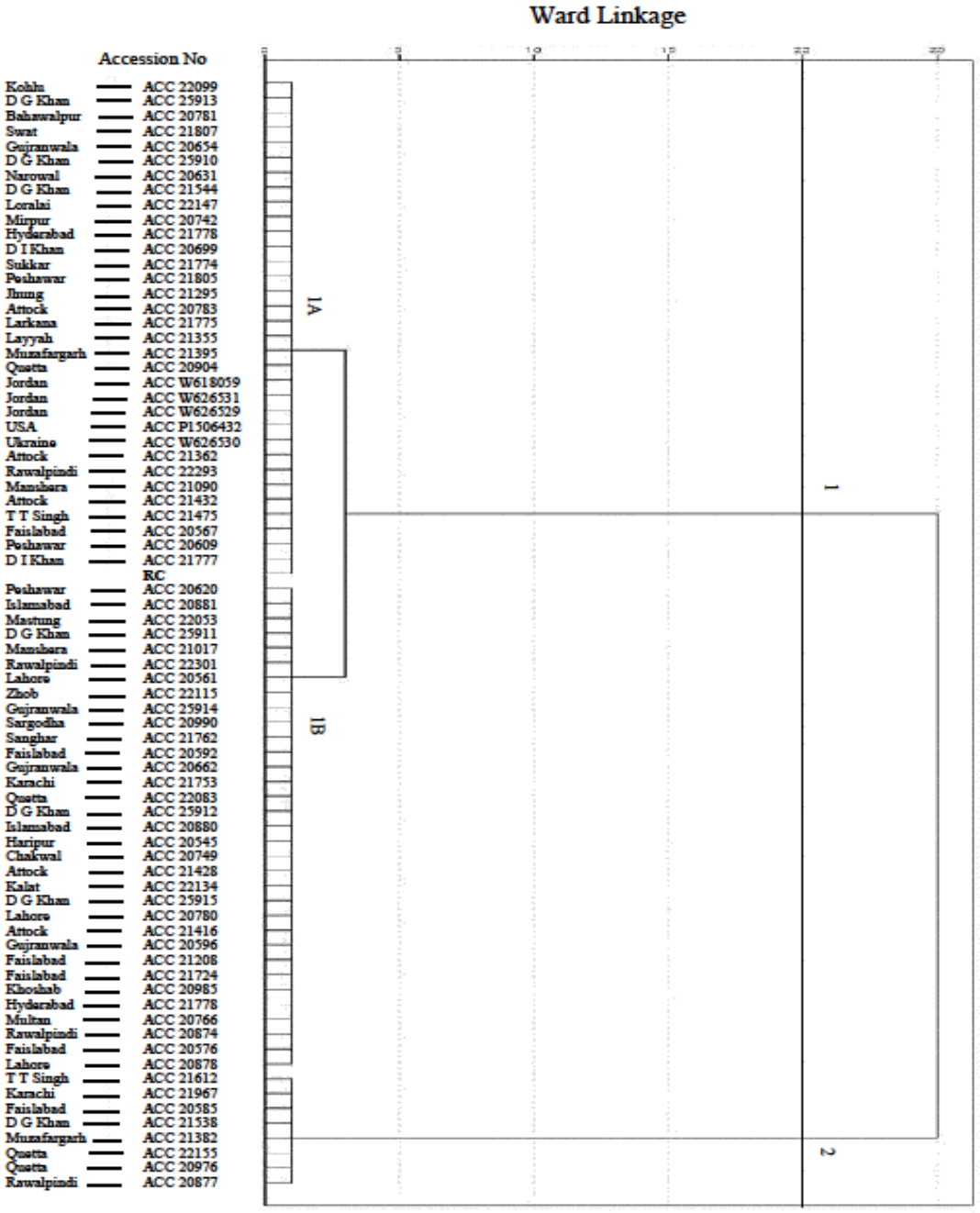

Fig. 6: Distribution of various accessions among clusters through Ward linkage for 2017-18

Discussion

Field morphological study in its pure form has never been widely practiced in many regions of the world being lengthy, labor intensive and environment susceptible technique. Although it can help the plant breeders to get estimate of morphological and molecular variations among crops. [13,14] Pharmacological properties have been traditionally attributed to *N. sativa* seeds, simply as a crushed powder, or as an extract. [17,18] Purified or as a mixture, metabolites of *N. sativa* seeds would present a potent and therapeutically interesting activity on the cardiovascular, respiratory, immune, and endocrine systems. [19] The present study was experimented for consecutive three years to estimate the exact phenotypic diversity among *N. sativa* germplasm. [20] reported that variation in biochemical components of plant accessions may be because of genetics, geographic locality and environmental fluctuations. It is assumed that the improvement in cultural practices may influence the phenotypic expression of crops like enhanced crop yield. [13,15,21] The characterization of morphological markers is a key step towards genetic diversity and phenotypic variability.[22]

Previous studies indicated that exploration of phenotypic diversity is based on a variety of morphological characters [12,23] similarly present study was designed in the same parameters to estimate the morphological variation among a number of morphological markers like plant height, number of branches, days to flower initiation, number of flowers, petals, stamens, follicles and seed per follicle. In the present study, most of the morphological markers like plant height, number of flowers, number of follicles and seed per follicle showed positive Pearson correlation among accessions. [12,24] used the same analytical approach to evaluate the correlation among different accessions of *N. sativa*and noticed that seed yield and biological yield showed the highest positive correlation. Some other markers like number of seeds per follicle and follicles per plant also showed positive correlation

The quantitative traits contribute directly towards economic importance of plants. [25] High variability among these quantitative characteristics strengthens the economic importance. [26] Likewise, in the present study twelve quantitative morphological markers were observed to estimate phenotypic diversity.*N. sativa* plant usually exhibited mixed (self / cross) pollination types but it is predicted that cross pollination may prove more fruitful with reference to genetic variation. [27] Similarly, in the present study the cross pollination of hundred individuals resulted in flower color variation and increase in plant height with more number of branches and follicles. In the present study it was observed that the recombinant produced by crossing two diverse international accessions W626529 (Jordan) and W626530 (Ukraine) belonging to two centers of diversity was comparatively bigger in plant height, better in number of branches, number of flowers, follicles and diverse in flower color. Similarly some other agriculturists advocated that hybrids are quite compatible as compared to their parents. [28]

The cluster analysis was done to assess the morpho-physiological parameters among accessions. The accessions belonging to different origin like Jordan, USA, Ukraine, Bahawalpur, Faisalabad and Karachi were found in the same sub-cluster. It may also predicted that same seed size may be because of their same seed contents like fatty acids, phenols and quinones. [29] On the other hand some of the results obtained among clusters were not consistent for three years that could be due to some environmental variables.[30] Some of the morphological markers like seed width and number of branches showed their negative correlation. [31,32] Some other researchers also noticed same pattern of negative Pearson correlation between seed size and seed yield among three varieties of *N. sativa*. [33]

In the present investigation, principle component analysis and cluster analysis showed distribution of whole

germplasm in two individual components PC1 and PC2. It is concluded from analysis that geographical distribution had no effect on germplasm distribution among components.[34] Similarly, a study in past showed almost uniform distribution of various genotypes of *B.napus* in the individual principle components (PC1/PC2) using PCA analysis and cluster distribution indicating less variability among accessions [12,35]

It was noted that morphological variations observed and collected for all three years were not consistent among all accessions, because of some environmental fluctuations but some of the accessions like ACC 20662, ACC W626529, ACC 20878, ACC 21545, ACC 21428and ACC 20990 showed consistency at some extent among results of different morphological markers (number of flowers, number of stamens, number of follicles, follicle length, follicle width and seed per follicle). In general the accessions belonging to Gujranwala, Jordan, Lahore, Haripur, Attock and Sargodha were found morphologically significant and diverse. The data collected from these accessions could be helpful in future for plant breeders to introduce new varieties and hybrid.

Conclusion

The accessions grown for consecutive three years showed diversity in the morphological markers. It was noticed that international accessions collected from Jordan and Ukraine were also quite diverse. Most of the morphological markers like plant height, days to flower initiation, and number of petals and stamens exhibited positive correlation among markers while seed length and tentacles number were negatively correlated with others. Cluster analysis showed a uniform distribution pattern of both the local as well as international accessions among sub-clusters and pretended that geographical distribution had no significant effect on diversity. Principal component analysis also showed uniform distribution of accessions among all the components and it was noticed that geographically distant accessions (Jordan, Ukraine, Layyah, US Idaho, Muzaffargarh and Quetta) were found in the same component. The recombinant (RC) plant showed increase in height with more number of branches and flower color variation. Hence, it is concluded that the recombinant plant and its parents (W626529/W626530) are highly recommended to plant breeders in future.

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تقييم التغير المورفولوجي للجراثيم في جميع أنحاء العالم من النباتات الهامة الصيدلانية *Nigella Sativa L*.

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ملخص

N. sativa هو محصول صناعي مهم على الصعيد العالمي ولكن المحاصيل المهملة في باكستان، وبالتالي المستوردة. وقد استخدم لتنشيط نظام الجسم في جميع الحضارات القديمة تقريبا. في هذا التحقيق في جميع أنحاء العالم تمت زراعة انضمام *N. sativa* لمدة ثلاث سنوات لاختيار الانضمامات الأكثر تنوعا للزراعة والغرض من التربية في المستقبل في باكستان. ولوحظت حالات انضمام أكثر تنوعا وتأقلما مع سمات مورفولوجية معززة. وأظهر طول البذور وعرض البذور وارتفاع النبات وعدد الزهور وعدد من بصيلات أقصى قدر من الاختلاف الكبير في ثلاث سنوات. ولم تكن الاختلافات الشكلية التي لوحظت وجمعت طوال السنوات الثلاث متسقة بين جميع حالات الانضمام، بسبب بعض التقلبات البيئية في حين أن بعض الانضمامات مثل لجنة التنسيق الإدارية 20662 ، لجنة التنسيق الإدارية W626529 ، لجنة التنسيق الإدارية 20878 ، لجنة التنسيق الإدارية 21545 ، لجنة التنسيق الإدارية 21428 و20990 ACC أظهرت الاتساق في حد ما بين نتائج علامات مورفولوجية مختلفة (عدد من الزهور ، وعدد من stamens ، وعدد من بصيلات ، طول المسام وعرض المسام والبذور لكل بصيلات). 50٪ الانضمامات انبت في 26–35 يوما على التوالي ثلاث سنوات وأظهرت النمو الأمثل. وقد لوحظ أن الارتفاع الأقصى هو 108 سم في انضمامين ACC 20878 و20780 ACC. لوحظ الحد الأقصى لتواتر أيام بدء زهرة 101–110 يوما. ولوحظ أن توزيع التردد الأقصى لعدد الفروع في فئة التردد 11–15 خلال السنوات الثلاث كلها. لوحظ الحد الأقصى لتواتر أيام بدء الزهور 101–110 يوما للفترة 2015–2016 و2017–2018. وأظهرت البيانات المسجلة في 2017–2018 الحد الأقصى لعدد الزهور على أنه 15 (20780 ACC و20878 ACC). وبلغ معدل إنتاج الجريبات لكل مصنع 10 من بين حالات الانضمام (W626529 ACC، و20545 ACC، و ACC 21295). وكانت أكبر فئة تردد لعدد stamens 21-25 stamens ، في حين أن النباتات مع تردد فئة 31–35 stamens كانت أقل تواترا. تنوع لون الزهرة في النباتات المؤتلفة (RC) كما الأبيض الأرجواني في حين أنه في الآباء كان الأبيض (الأردن) والأبيض المزرق (أوكرانيا). بشكل عام تم العثور على الانضمامات التي تنتمي إلى غوجرانوالا والأردن ولاهور وهاريبور وأتوك وسارغودا أكثر أهمية وتنوعا من الناحية الشكلية.

الكلمات الدالة: المجموعة؛ الجرثومة، مورفولوجيا، سكان.

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