Morphological Characterization and Diversity Analysis of Adzukibean [*Vigna angularis* (Willd.) Ohwi and Ohashi] Germplasm

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A B S T R A C T

Keywords	
characterization, Clustering, Adzuki bean, Diversity	One hundred and fifty-one genotypes of adzuki bean were evaluated at the experimental farm of the Department of Genetics and Plant Breeding, CSKHPKV, Palampur in Augmented Block Design during *Kharif*, 2019 for characterization and diversity analysis using 22 agro-morphological characters. 5 genotypes viz., EC-340286, EC-340267, EC-340271, EC-000263 and EC-340251 showed resistance to *Cercospora* leaf spot disease. Cluster analysis grouped these genotypes into ten clusters with cluster III having highest 36 genotypes. Sufficient variability was observed in the genotypes studied based on characterization and cluster analysis which could be utilized in breeding programmes of this crop.
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Introduction

Adzuki bean [*Vigna angularis* (Willd.) Ohwi and Ohashi] is a self-pollinated legume crop (2n=22) belonging to the genus *Vigna* of thefamily *Leguminosae*. Its center of origin is East Asia andit is mainly cultivated in China, Japan, Taiwan, South Korea, Bhutan, India, Nepal, US, South America, New Zealand and Africa. *V. angularis* var. *nipponensis* is the presumed wild ancestor of cultivated adzuki bean (Yamaguchi *et al.,* 1992). This wild species is distributed across a wide area of Japan, the Korean and China peninsula, including Taiwan, Nepal and Bhutan

(Vaughan *et al.,* 2004). China is the largest producer of adzuki bean in the world with an estimated production of about 260 thousand tons in the year 2019-20. In Japan, it is the second most important legume after soybean with a consumption of about 140,000 tonnes per year (Anonymous, 2019). Outside East Asia, Canada and Australia have also started producing adzuki bean in recent years, mainly for export reasons. In India, it is considered as an underutilized crop and its cultivation is limited to the North-eastern and Northern hill zones. It occasionally grows in Chamba, Kangra, Mandi and Bilaspur districts of Himachal Pradesh where it is mainly grown

as an intercrop with maize (Shweta, 2013). It is an annual vine with trifoliate leaves, yellow flowers, slender and curved pods, determinate and late maturing. Its seed coat colour is generally wine red but black, white, yellow or mottled colour also exists. It is consumed in the form of whole seed, split grain and flour while, immature pods and leaves are eaten as vegetable (Dua *et al.,* 2009). In India, it is taken as a pulse crop but in Japan and China it is widely used in a variety of foods like cake, porridge, desserts, adzuki rice, jelly, adzuki milk and ice cream. It is an important legume crop of temperate and sub temperate regions grown for human consumption due to its high nutritional values and broad adaptability. It improves soil condition by nitrogen fixation up to the tune of 100 kg/ha. It is thus evident that adzuki bean has the potential of being an agriculturally important crop and has large scope in future to meet the demand of pulses.

The low genetic variability observed in the crop demands the strengthening of breeding programmes through introduction of new germplasm, collection of local genotypes and adopting interspecific hybridization. So, there is an urgent need to characterize and evaluate adzuki bean genotypes to identify donor(s) for different traits and utilizing these genotypes in different breeding programmes. Germplasm plays the key role in providing needed attributes for developing superior varieties. Characterization involves estimating existing variability across the population of individuals (Franco and Hidalgo, 2003). Describing the characteristics of a crop species based on standard descriptors is helpful for better utilization and conservation of germplasm (Kumari *et al.,* 2017). Moreover, morphological characterization studies play an important role in better understanding of crop diversity. Thus, it is essential to conserve the genotypes and explore the gene pool of adzuki bean for breeding purposes. Diversity analysis is an

essential process for identification of the genetic relatedness of the available genetic resources and to display the trends associated with latitude of germplasm origin, which were positive or negative according to the trait (Wang *et al.,* 2001). Mahalanobis D^2-analysis (1936) is a very useful tool in studying the nature and magnitude of diversity present in the available germplasm. It helps in the selection of diverse parental combinations to produce segregating progenies with maximum genetic variability (Barrett and Kidwell, 1998) and introgressing desirable traits from diverse germplasm to cultivated varieties to broaden their genetic base (Thompson *et al.,* 1998). Therefore, present investigation was undertaken for morphological characterization studies and to identify parents for future crop breeding programme for the improvement of this underutilized pulse.

Materials and Methods

The experimental material for the present study comprised of 151 adzuki bean genotypes along with 2 checks viz., Local Totru and HPU-51, evaluated at the experimental farm of Department of Genetics and Plant Breeding, CSKHPKV, Palampur during *Kharif*, 2019 for 22 agro-morphological characters including 10 quantitative and 12 qualitative traits in Augmented Block Design (Federer, 1956) during *Kharif*, 2019 (Table 1). Each entry was raised in one rowed plot of 3m length with row to row and plant to plant spacing of 30cm and 10cm, respectively in 15 blocks. Each block consisted of 12 genotypes including 2 checks and the checks were allotted randomly in each block. The crop was raised following standard package of practices. Data was recorded on five randomly choosen plants for the traits *viz.,* plant height (cm), primary branches per plant, clusters per plant, pods per cluster, pods per plant, seeds per pod, 1000-seed weight (g) and seed yield per plant (g)

while, for days to 50% flowering and days to 80% maturity data was recorded on plot basis. Data was also recorded for the qualitative traits *viz*., early plant vigour, plant habit, leaf colour, leaf surface, leaflet shape, flower colour, stem colour, stem surface, pod angle, pod surface, seed coat colour and biotic stress susceptibility. Grouping of genotypes according to resistance against *Cercospora* leaf spot disease was done using 0-9 scale as per Alice and Nadarajan (2007).Genetic diversity studies were conducted as per Mahalanobis D^2-analysis (1936).Frequency distribution of these traits was performed using StatistiXL version 1.10 software to determine the best relationships among characters and genotypes.

Results and Discussion

Frequency distribution of morphological traits

Qualitative traits were studied on the basis of visual observations and their frequency distribution revealed the presence of significant variation in adzuki bean germplasm [Fig. 1(a)-1(l)]. 77.8% of the total accessions had good nature of growth followed by 15.7% having very good plant vigour for the character early plant vigour [Fig. 1(a)]. All accessions i.e. 100% showed indeterminate type of growth habit [Fig. 1(b)]. Three types of leaf colour were observed in the germplasm i.e. yellowish green, green and dark green. 52.3% plants were having green coloured leaves followed by 38.6% which were having yellowish green leaves [Fig. 1(c)]. Most of the accessions, 69.3% were having pubescent type of leaf surfaces [Fig. 1(d)]. About 63.4% accessions had entire shape of leaflet, while 34% were having lobed shaped leaflet [Fig. 1(e)]. For stem pigmentation, 94.8% of accessions were found to have green coloured stem [Fig. 1(f)]. 90.2% genotypes were having pubescent type

of stem surface whereas, only 9.8% showed glabrous type of stem surface [Fig. 1(g)]. Regarding flower colour92.8% accessions were having yellow coloured flowers while, 7.2% were having light yellow coloured flowers [Fig. 1(h)]. All accessions i.e. 100% were having erect type of pod angle [Fig. 1(i)]. Maximum number of accessions i.e. 96.1% had glabrous pod surface [Fig. 1(j)]. Seeds of adzuki bean germplasm have shown wide variation with respect to seed coat colour. Maximum accessions i.e. 80.4% possessed red seed coat colour, followed by 17.6% which showed other types of seed colour and 2% were having maroon type of seed coat colours [Fig. 1(k)]. 60% of the accessions were susceptible to the disease while, 31.4% had moderate susceptibility. Only 2% accessions showed resistance to the disease whereas, 2.6% accessions were moderately resistant to the disease.[Fig. 1(l)].

Disease reaction

Response to *Cercospora* leaf spot disease was observed among the accessions under open field condition (Table 2). Results revealed that 5 genotypes viz., EC-340286, EC-340267, EC-340271, EC-000263 and EC-340251 showed resistance to the disease. 9 genotypes viz., EC-000377, EC-340284, IC-341960, EC-57959, IC-485385, EC-340277, IC-341951, IC-34193 and IC-341963 were found moderately resistant, 50 genotypes were found moderately susceptible, 85 were found susceptible and 4 genotypes were showing very high susceptibility to the disease.

Resistant genotypes can be used as donor parents for future breeding programme. Kaushal and Singh (1988) introgress the resistance of adzuki bean against fungal and viral diseases into urdbean by crossing them to achieve an interspecific hybrid using different non-conventional breeding methods.

Table.1 List of germplasm lines of adzuki bean evaluated under the present study

S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes
1	EC-290251	41	EC-24102	81	EC-000271	115	EC-34264
2	EC-187896	42	EC-341943	82	EC-340277	116	EC-36070
3	IC-341948	43	EC-340276	83	IC-469174	117	EC-18959
4	EC-340272	44	EC-340280	84	IC-341944	118	IC-46917
5	EC-340258	45	EC-59459	85	EC-120480	119	EC-240251
6	EC-15648	46	EC-340279	86	IC-241041	120	EC-281186
7	EC-340286	47	EC-340259	87	EC-120466	121	IC-485396
8	EC-120460	48	EC-30250	88	IC-469172	122	EC-36973
9	IC-341957	49	EC-340267	89	EC-87896	123	IC-30270
10	EC-340247	50	IC-485886	90	EC-340246	124	IC-341940
11	IC-341951	51	EC-187898	91	IC-341956	125	IC-24105
12	EC-340274	52	EC-340268	92	EC-341955	126	IC-24522
13	EC-59489	53	EC-000262	93	IC-341952	127	EC-000263
14	EC-340281	54	EC-340249	94	EC-340287	128	EC-57959
15	EC-240246	55	EC-34027	95	IC-341939	129	EC-340269
16	IC-251353	56	EC-340283	96	IC-341947	130	IC-341942
17	EC-340275	57	EC-340248	97	EC-340278	131	IC-0293
18	IC-341950	58	EC-87071	98	EC-340251	132	IC-140846
19	IC-341953	59	EC-340270	99	EC-340244	133	IC-34196
20	EC-340245	60	IC-469175	100	IC-89957	134	IC-140845
21	IC-341949	61	EC-340264	101	IC-108855	135	EC-34625
22	EC-340257	62	IC-485385	102	EC-340256	136	EC-18151
23	EC-340265	63	EC-340252	103	IC-341954	137	EC-341962
24	EC-000248	64	EC-000276	104	IC-341938	138	EC-340266
25	IC-108856	65	EC-15256	105	IC-341937	139	IC-341959
26	EC-000254	66	EC-340250	106	EC-87815	140	IC-341960
27	EC-340240	67	EC-340263	107	EC-87895	141	EC-340260
28	EC-57159	68	EC-30253	108	IC-140848	142	EC-340262
29	EC-340254	69	EC-30270	109	IC-485382	143	EC-340273
30	EC-000249	70	EC-340261	110	IC-485388	144	EC-340255
31	EC-290652	71	EC-340288	111	EC-18256	145	IC-108857
32	IC-341958	72	EC-108080	112	IC-341941	146	EC-57459
33	IC-108854	73	EC-24523	113	IC-341946	147	IC-455396
34	EC-340285	74	EC-08707	114	EC-000372	148	EC-340271
35	EC-000251	75	IC-16761	109	IC-485382	149	EC-80850
36	EC-340284	76	IC-341945	110	IC-485388	150	EC-000264
37	EC-340282	77	IC-469173	111	EC-18256	151	EC-15257
38	EC-340253	78	IC-341963	112	IC-341941	152	Local Totru (c)
39	EC-000377	79	EC-30256	113	IC-341946	153	HPU-51 (c)
40	IC-100072	80	EC-18257	114	EC-000372		

Source/Pedigree: NBPGR, Shimla (H.P.)

Table.2 Reaction of adzuki bean genotypes to *Cercospora* leaf spot disease under natural conditions

Disease incidence (%)	Rating scale	Reaction	No. of genotypes	Genotypes
0	0	Highly resistant	-	-
1-5%	1	Resistant	5	EC-340286, EC-340267, EC-340271, EC-000263, EC-340251
6-10%	3	Moderately resistant	9	EC-000377, EC-340284, IC-341960, EC-57959, IC-485385, EC-340277, IC-341951, IC-341937, IC-341963
11-25%	5	Moderately susceptible	50	EC-000251, EC-340282, IC-16761, Local Totru, HPU-51, IC-46917, EC-340276, EC-34027, EC-240251, IC-24105, EC-000276, EC-15256, EC-340250, EC-340263, EC-30253, EC-30270, EC-340261, EC-08707, IC-469173, IC-469174, IC-341944, EC-120480, IC-469172, EC-87896, EC-340246, EC-340287, IC-341939, IC-341947, EC-340256, EC-87815, EC-87895, IC-140848, IC-341946, EC-341962, EC-340266, IC-341959, EC-57459, IC-455396, EC-15257, IC-341940, EC-290251, IC-251353, IC-341953, EC-000248, IC-108856, EC-000254, EC-340240, EC-57159, EC-340254, EC-000249
26-50%	7	Susceptible	85	EC-187896, IC-341948, EC-340272, EC-340258, EC-15648, EC-281186, EC-120460, IC-341957, EC-340275, IC-341950, EC-340245, IC-341949, EC-340257, EC-340265, EC-290652, IC-341958, IC-108854, EC-340285, EC-340253, IC-100072, EC-24102, EC-341943, EC-340280, EC-59459, EC-340247, EC-340279, EC-340259, EC-30250 IC-485886, EC-187898, EC-340268, EC-000262, EC-340249, EC-340283, EC-340248, EC-87071, EC-340270, IC-469175, EC-340264, EC-340252, EC-340288, EC-108080, EC-24523, IC-341945, EC-30256, EC-18257, EC-000271, IC-241041, EC-120466, IC-341956, EC-341955, IC-341952, EC-340278, EC-340244, IC-89957, IC-108855, IC-341954, IC-341938, IC-485382, IC-485388, EC-18256, IC-341941, EC-000372, EC-34264, EC-36070, EC-18959, IC-485396, EC-36973, IC-30270, EC-340269, IC-341942, IC-0293, IC-140846, IC-34196, IC-140845, EC-34625, EC-18151, IC-24522, EC-340260, EC-340262, EC-340273, EC-340255, IC-108857, EC-80850, EC-000264
>50%	9	Highly susceptible	4	EC-340274, EC-59489, EC-340281, EC-240246

Table.3 Clustering pattern of 153 adzuki bean genotypes according to Mahalanobis D2- analysis

Clusters	Number of genotypes	Genotypes
1	6	EC-000254, IC-341957, EC-59459, EC-34027, EC-30253, IC-485382
II	18	EC-187898, IC-341950, IC-485886, EC-340267, EC-340280, IC-485396, EC-30250, EC-340248, EC-341943, EC-120480, EC-340257, IC-341952, IC-341945, EC-340287, IC-341947, EC-340283, EC-30270, IC-469172
III	36	EC-340269, EC-36070, EC-340288, EC-340256, EC-340244, IC-341942, IC-24522, EC-120466, EC-34264, EC-340259, EC-340260, EC-340275, IC-140848, EC-340240, EC-30256, IC-251353, EC-87815, IC-341951, EC-24102, EC-000262, IC-108854, EC-187896, IC-341939, EC-340251, HPU– 51, IC-455396, EC-340250, Local Totru, IC-341940, EC-340265, EC-000264, IC-30270, IC-341949, IC-34196, EC-87895, EC-340274
IV	14	EC-15257, EC-340245, EC-57959, EC-87896, EC-340273, EC-57159, EC-340282, IC-341953, EC-34625, IC-16761, EC-340278, EC-340246, IC-341956, EC-341955
V	24	IC-341954, IC-341938, IC-341937, EC-18257, EC-000249, EC-290652, IC-341959, EC-15256, EC-36973, EC-000248, EC-000372, IC-341944, IC-241041, EC-340249, IC-485385, EC-341962, EC-340268, EC-340255, EC-57459, IC-108855, EC-340266, IC-140846, IC-108856, IC-469173
VI	4	EC-80850, IC-89957, IC-0293, EC-340279
VII	33	EC-87071, EC-340258, IC-46917, IC-341948, EC-340270, EC-59489, EC-340272, IC-24105, EC-120460, EC-240251, EC-000276, EC-340271, EC-000377, EC-15648, EC-340247, EC-290251, EC-340252, EC-340281, IC-108857, EC-340285, EC-240246, EC-18151, EC-340286, EC-340276, IC-341941, IC-100072, EC-340264, EC-18959, IC-341958, IC-341946, EC-340253, EC-000251, EC-340254
VIII	3	EC-281186, IC-469175, EC-340284
IX	5	EC-000271, EC-340277, EC-000263, EC-340262, IC-341960
X	10	IC-140845, EC-24523, EC-340263, IC-469174, EC-340261, IC-485388, IC-341963, EC-18256, EC-108080, EC-08707

Table.4 Average intra and inter cluster distances

Inter cluster distance:

	Cluster 1 31.72	Cluster 2 24.91	Cluster 3 30.1	Cluster 4 29.25	Cluster 5 25.65	Cluster 6 25.87	Cluster 7 27.14	Cluster 8 25.13	Cluster 9 33.34	Cluster 10 28.66
Cluster 1	0.00	55.45	45.28	88.75	40.05	56.28	52.25	52.16	36.42	41.07
Cluster 2		0.00	66.29	107.34	40.24	70.96	66.92	62.03	60.53	42.29
Cluster 3			0.00	74.03	44.69	45.90	34.21	46.05	40.10	60.11
Cluster 4				0.00	88.73	54.73	72.47	91.60	73.60	103.75
Cluster 5					0.00	56.28	46.53	49.62	44.91	41.22
Cluster 6						0.00	40.73	66.91	43.30	72.85
Cluster 7							0.00	55.68	47.44	62.72
Cluster 8								0.00	57.79	47.80
Cluster 9									0.00	53.26
Cluster 10										0.00

Table.5 Mean values of ten clusters for different traits of adzukibean genotypes

	Days to 50% flowering	Days to 80% maturity	Plant height	Primary branches/ plant	Clusters/ plant	Pods/cluster	Pods/plant	Seeds/pod	1000-seed weight	Seed yield/plant
Cluster 1	69.77	108.72	88.72	3.87	14	2.79	27.94*	7.54	83.93	12.17
Cluster 2	64.66*	106.83	95.27**	3.39	15.64	3.22	39.59	7.69	67.94*	12.94**
Cluster 3	65.54	106.47	76.65	3.51	16.30	3.03	37.65	7.25	99.13	12.11
Cluster 4	69.52	113.17	84.14	3.68	13.69*	3.17	34.97	7.70	146.15**	12.94**
Cluster 5	64.85	107.63	85.10	3.36	17.55	2.86	39.39	7.53	83.56	12.74
Cluster 6	66.74	107.61	89.16	2.87*	17.82	3.41**	39.38	7.46	118.12	12.35
Cluster 7	65.41	104.31*	83.67	3.39	18.99**	3.31	50.64**	6.91*	106.27	11.35
Cluster 8	66.27	106.17	62.47*	3.40	13.74	2.15*	33.76	7.36	83.19	10.95*
Cluster 9	69.34	110.80	87.67	3.60	14.88	2.94	33.24	7.78	96.39	12.08
Cluster 10	72.58**	116.60**	83.10	4.42**	13.85	2.57	31.93	8.10**	71.18	12.72

**and *: maximum and minimum values respectively

Fig.1 Frequency distribution of adzukibean germplasm for qualitative traits
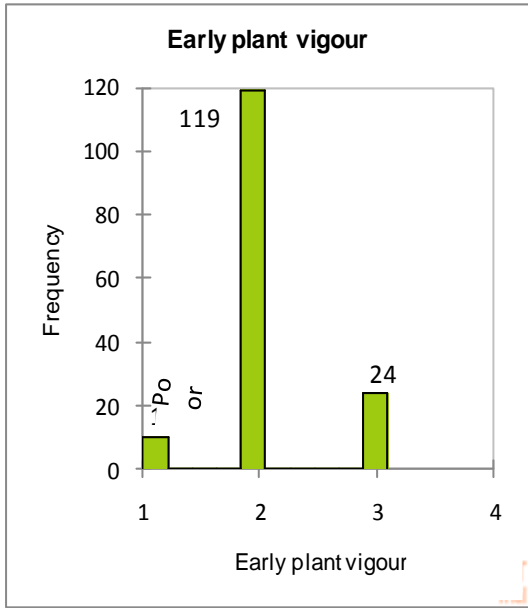
Fig.1(a)

Fig.1(b)

Fig.1(c)

Fig.1(d)

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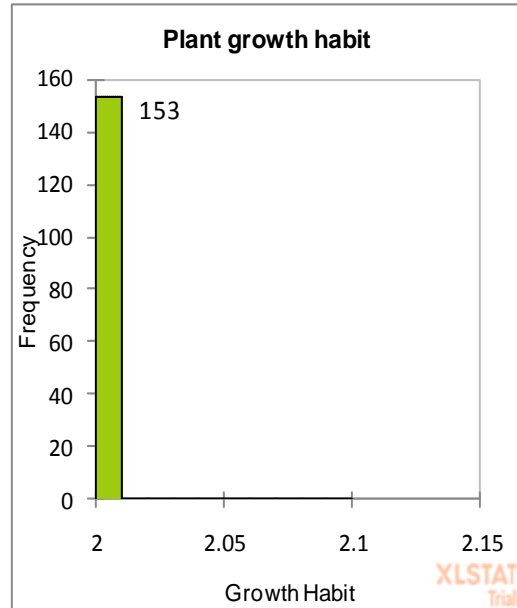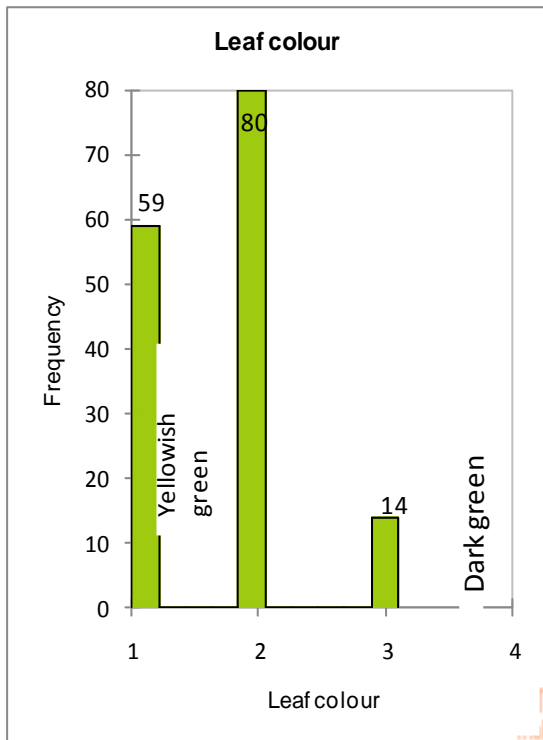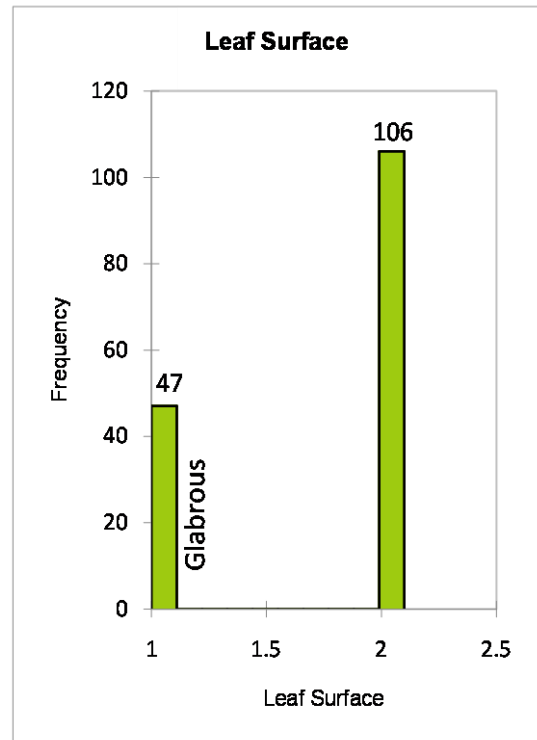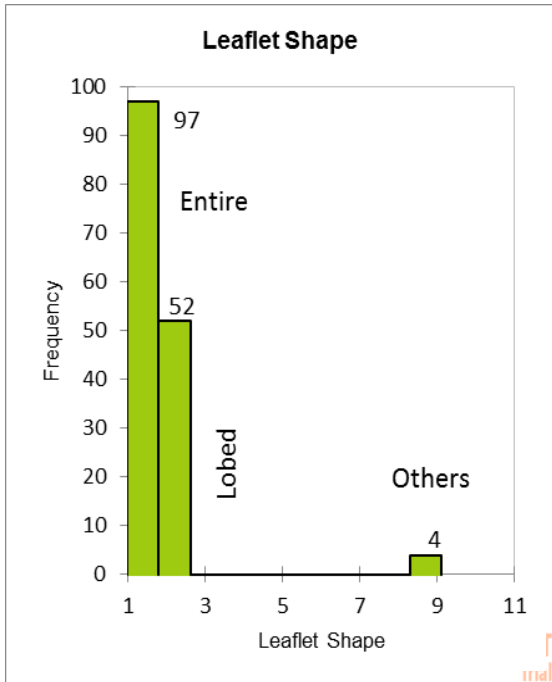
Fig.1(e)

Fig.1(f)

Fig.1(g)

Fig.1(h)

612
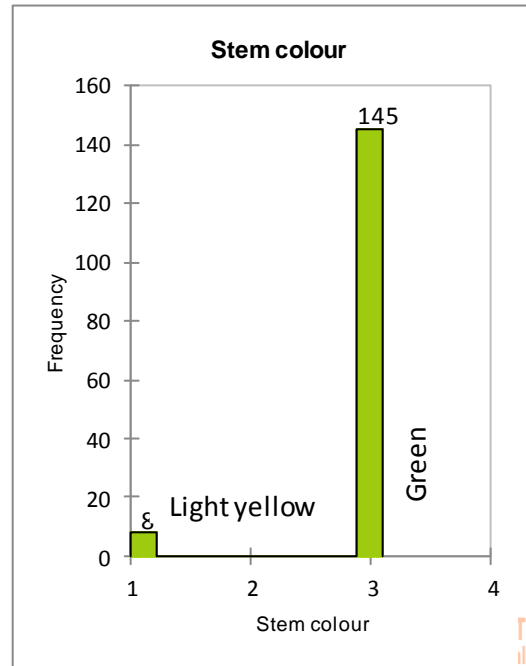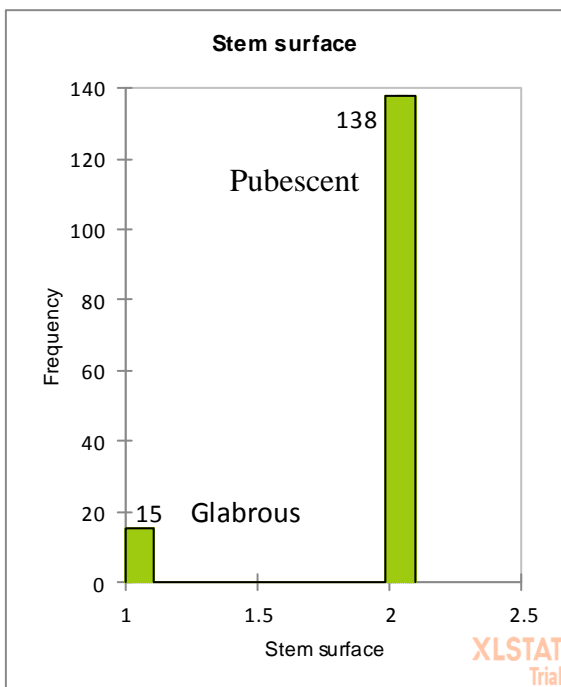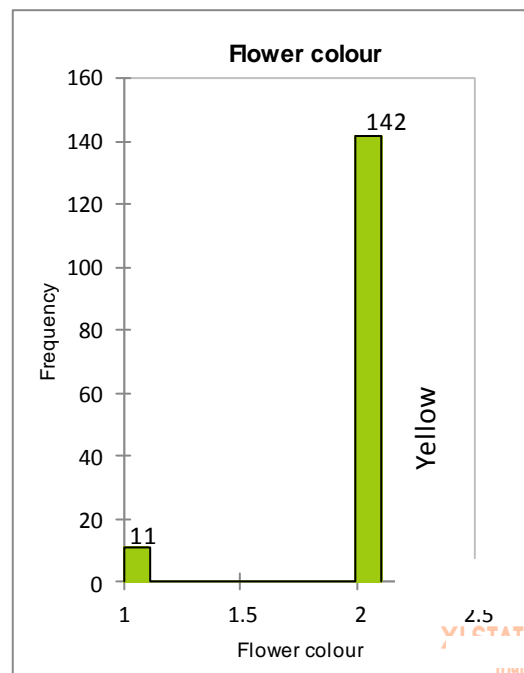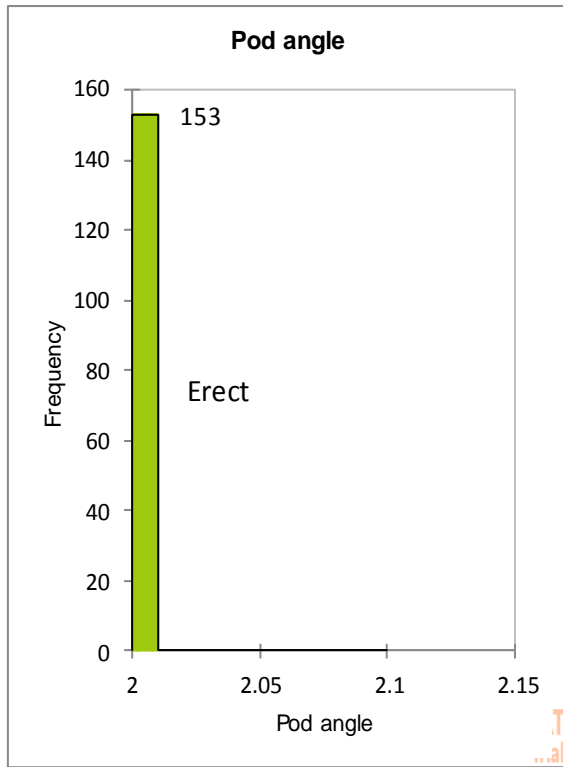
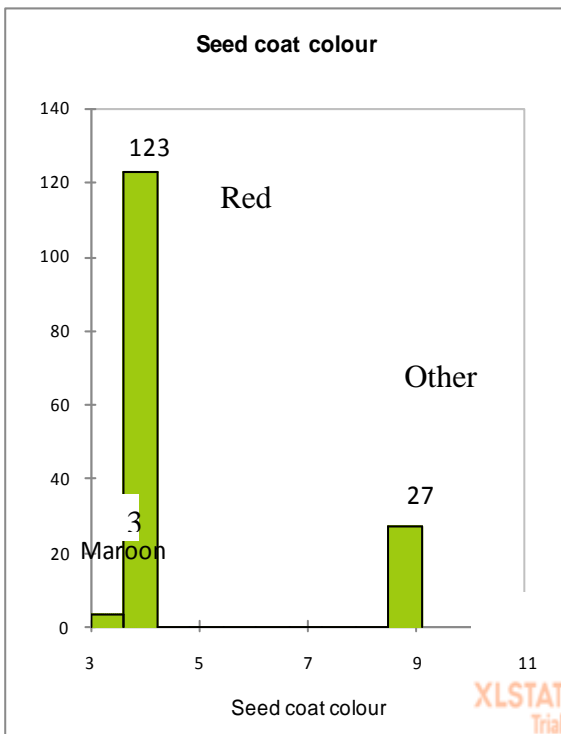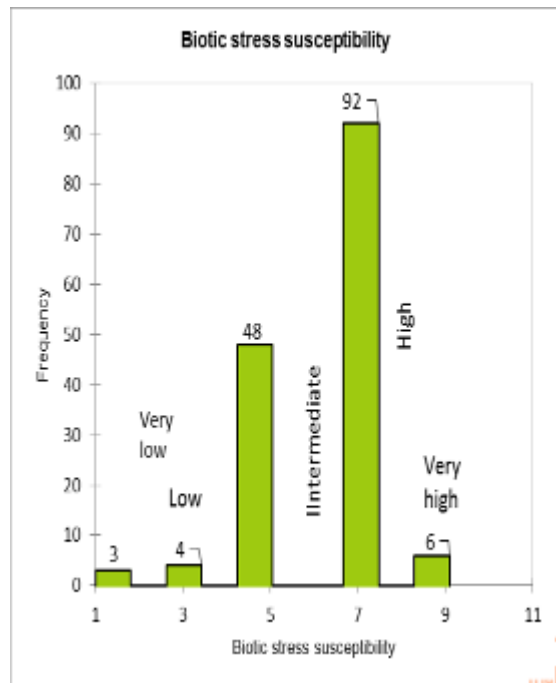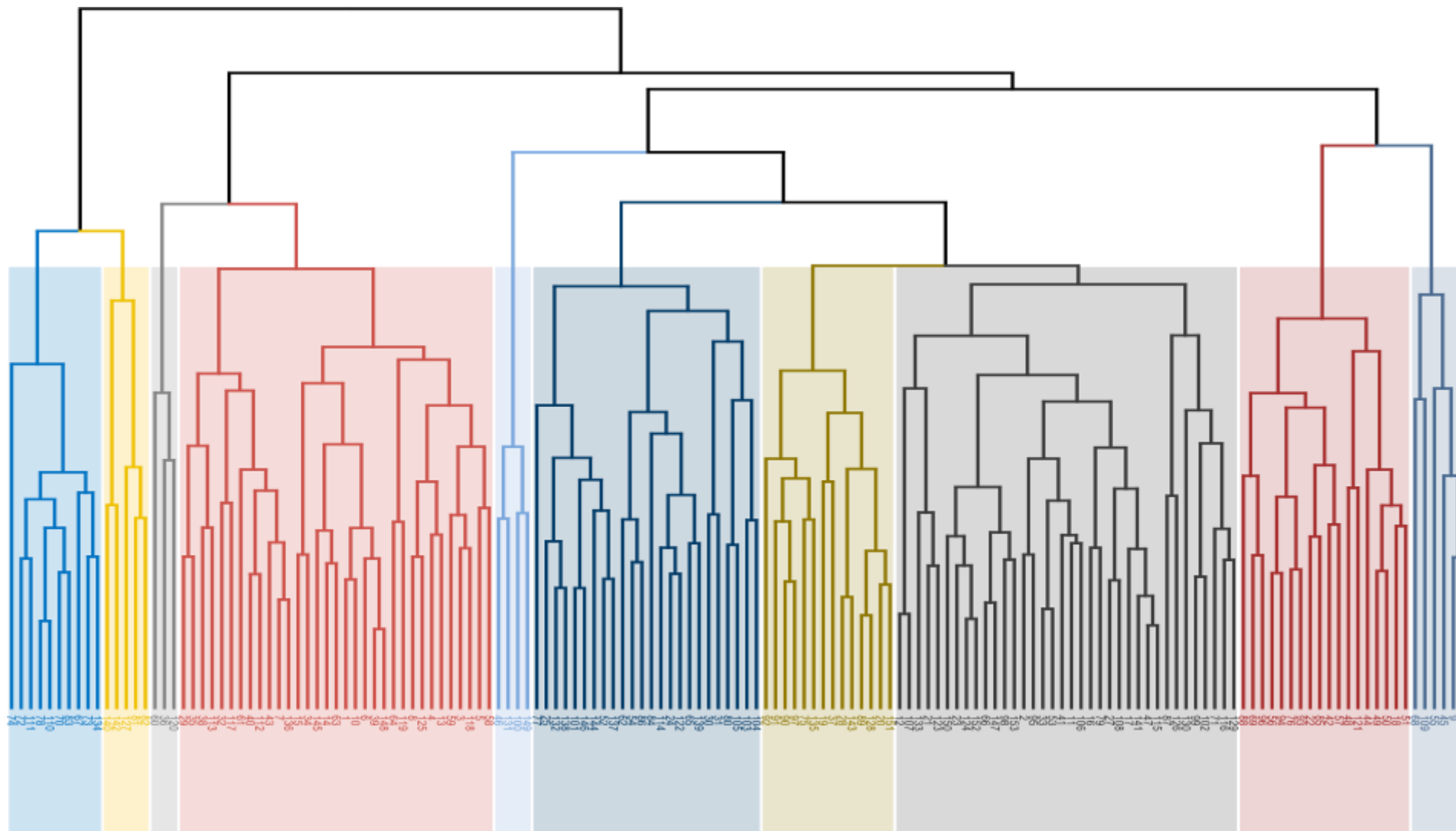**Fig.1(i)**
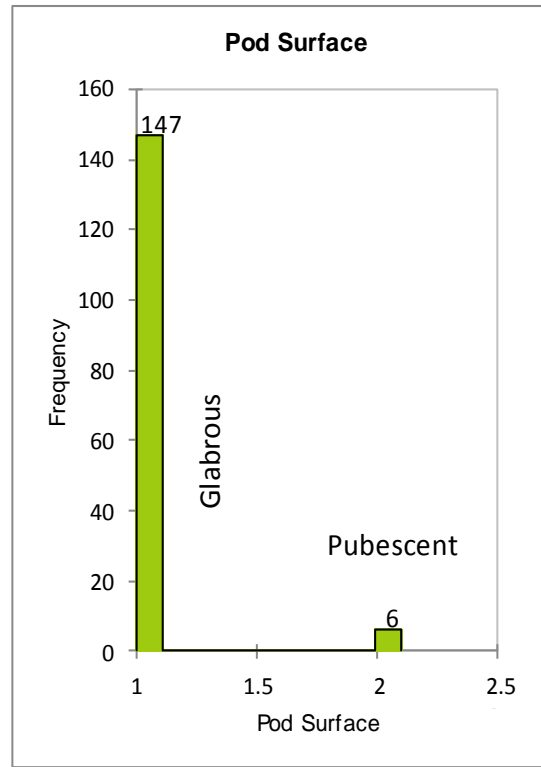
Fig.1(j)

Fig.1(k)

Fig.1(l)

Fig.2 Dendrogram depicting relationships among the adzuki bean genotypes

Cluster analysis

Non-hierarchical Euclidean cluster analysis grouped 153 genotypes of adzuki bean including checks into ten clusters with cluster III having highest number of genotypes i.e. 36, followed by cluster VII with 33 genotypes and cluster V with 24 genotypes (Table 3 and Fig. 1). Average intra and inter cluster distances are presented in Table 4. Different clustering patterns were also reported in adzuki bean by some earlier workers (Zhen-xing, 2011; Redden *et al.,* 2009; Wang *et al.,* 2019).

Maximum intra cluster distance was observed in cluster IX (33.34) followed by cluster I (31.72) indicating genetic diversity among the genotypes belonging to these clusters. Therefore, the crosses should be made between the genotypes of these clusters for developing good segregants. The maximum inter cluster distance was observed between cluster II and cluster IV (107.34) followed by distance between cluster IV and cluster 1X (103.75) suggesting wide diversity between these clusters. Therefore, genotypes belonging to these clusters may be used in hybridization programme for the improvement of adzuki bean. The least inter-cluster distance was observed between cluster III and VII (34.21) indicating close relationship between the genotypes of these clusters.

The diversity was also supported by the considerable amount of variation among the cluster mean for different characters. The cluster means of adzuki bean genotypes falling under different clusters are presented in Table 5. Cluster 2 showed the maximum cluster mean values for plant height and seed yield per plant. For 1000-seed weight and seed yield per plant, cluster 4 recorded the maximum values. Cluster 6 showed the maximum values for pods per cluster.

Maximum values for pods per plant and clusters per plant were recorded by cluster 7. Cluster 10 showed the maximum cluster mean values for days to 50% flowering, days to 80% maturity, primary branches per plant and number of seeds per pod. Thus, these genotypes hold great promise as parents for procuring elite lines through hybridization and to create further variability for these characters.

Diversity analysis is an essential process for identification of the genetic relatedness of the available genetic resources which is helpful in better utilization and conservation of germplasm. Frequency distribution graphs of various qualitative traits revealed sufficient variation among adzuki bean accessions and provided useful information about the diversity of these accessions. Reaction of adzuki bean to other diseases like brown stem rot, charcoal rot Fusarium wilt etc. was studied in China by many scientists but studies related to disease incidence and reaction of adzuki bean to *Cercospora* leaf spot is not much done. Here in this study, response to *Cercospora* leaf spot disease showed that 5 genotypes viz., EC-340286, EC-340267, EC-340271, EC-000263 and EC-340251 were resistant to the disease.

In the present study, sufficient variability was observed in adzuki bean accessions. Cluster analysis clearly showed that some genotype is closely related while some as significantly distinct. 153 adzuki bean genotypes including checks were grouped into ten clusters with cluster III having highest number of genotypes i.e. 36, followed by cluster VII and cluster V. Maximum intra cluster distance was observed in cluster IX (33.34). The maximum inter cluster distance was observed between cluster II and cluster IV (107.34) indicating genetic diversity among the genotypes belonging to these clusters. Therefore, crosses should be made between

these genotypes for developing good segregants.

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