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D^2 Statistical Analysis of Tuber Yield and its Contributing Traits in Winged Bean [*Psophocarpus tetragonolobus* (L.) Dc.]

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ABSTRACT: Winged bean is a multipurpose protein rich underutilized pulse species which has a wider genetic potentiality, to be utilized as a source of quality food. But information regarding genetic diversity of winged bean tuber is limited. Therefore, present investigation was carried out to determine the genetic divergence for tuber yield and its contributing traits in twenty two genotypes of winged bean. The research trial was conducted at vegetable research farm of Banaras Hindu University, Varanasi, Uttar Pradesh during *kharif* season of 2015-2016 with three replications. Each plot had one row of 5m length with 50cm plant to plant and 75cm row to row distance. The vegetative & reproductive pruning were done to raise a good tuber yield. Observations on thirteen quantitative traits and four qualitative traits were recorded in five pruned plants from each genotype per replication. The replicated mean data was used in WINDOSTAT statistical package. The results revealed that, there was a significant (P<0.01) difference between genotypes for all the seventeen traits under studied. D^2 analysis partitioned the twenty-two genotypes of winged bean into five clusters, indicating the presence of genetic diversity. The cluster IV, II and I had the higher number of genotypes, 9, 6 and 5 respectively. Cluster III and V consist of only single genotype, indicating their distinctness among themselves and also from other genotypes. The higher inter-cluster distance was observed between clusters IV and V (519.48) and the higher intra-cluster distance was found in cluster IV (216.80). Cluster V exhibited maximum mean performance for days to pod initiation, days to tuber harvesting, tuber length, tuber breadth, tuber dry matter percent, total sugar content in tuber, tuber yield per plant. Intercrossing among the genotypes belonging to clusters IV and V was suggested to obtaining transgressive/desirable segregants for developing high yielding cultivars with better quality.

Keywords: Winged bean, Tuber, Genetic divergence, Clusters, Genotypes.

INTRODUCTION

The world population are increasing day by day with fluctuate climate, and millions of people in the world are suffering from malnutrition and hunger. However, mostly half of the world population consumed major three crops such as maize, rice and wheat, yet these cereal grains are relatively poor in quality protein and some micronutrients. Therefore, pulses complement cereals in the diet (Foyer *et al.,* 2016); they provide considerable protein with essential amino acids and micronutrients, and serve as a substitute for animal protein when this is unavailable (Tharanathan and Mahadevamma 2003). In addition to nutritious value, legumes have ability to fix atmospheric nitrogen and improve the soil fertility gradient for subsequent crops (Peoples *et al.,* 1995). Therefore, research is needed into a such type of plant species which has a wide genetic potentiality to fight against malnutrition and hidden hunger of the community and capable to grow under stressful or suboptimal conditions (Campbell *et al.,* 2016). These problems may be solved by some underutilized plant species. Several legumes crops are described as underutilized or orphan crops, *i.e.*, crops that are locally consumed, but essentially absent in international trade and never received any appreciable research effort (Cullis and Kunert 2017).

Winged bean [*Psophocarpus tertagonolobus* (L.) DC] is a such type of multipurpose protein rich underutilized legume species which has a wider genetic potentiality, to be utilized as a source of quality food under climate change decade (Sriwichai *et al*., 2021). According to Jane E. Brody (1982), winged bean hailed as a potent weapon against malnutrition. Winged bean is a twining annual as well as perennial herb belongs to the family Fabaceae. Chromosome number 2n=2x=18 have been recorded (Khan 1976, Haq and Smartt 1977, Harder 1992). Every part of winged bean such as tubers, young pods, leaves, flowers and seeds are edible and rich in protein, amino acids, oils, vitamins and minerals (Claydon 1978, Hettiarachchy and Kantha 1982). According to Land *et al.* (1981), its roots differ from soybeans and other legumes in that they generally do not require artificial inoculation with specific stains of Rhizobium for fixation of atmospheric nitrogen into the soil.

An extraordinary feature in many cultivars of winged bean is that it produces tuberous roots like sweet potatoes, but are much richer in protein (3-15percent) with combination of carbohydrate (27.2- 30.5 percent) than potatoes, sweet potato, cassava and yam (Karikari 1978; Chandel and Pant 1984; Adegboyega *et al*. 2019). Its tubers have a pleasant and slightly sweet taste, ivory white flesh and firm texture resembling an apple. It can be consumed by boiled, steamed, fried, roasted and made into chips. The winged bean tubers are a very popular and highly acceptable food in Myanmar, Indonesia and Northern Ghana village population (Karikari 1978; Sriwichai *et al*. 2021). In India also tribal people in Tripura, Manipur, Mizoram and some adjoining areas of Assam, consume tuberous roots of winged bean in their diet.

Although, very little information available regarding divergence in winged bean germplasm especially for tuber producing genotypes. Until now winged bean never received any appreciable research effort and funding, especially for its yield improvement. Wide genetic diversity is the pre-requisite tools for genetic improvement of any crop and helps the plant breeder in choosing the diverse parents for purposeful hybridization programme. Therefore, realizing the importance and present need of this underutilized legume, the present experiment was conducted to study the genetic divergence (D^2 analysis) and clustering pattern in twenty two winged bean genotypes.

MATERIALS AND METHODS

The experimental material consists of twenty two genotypes of winged bean. These were collected from Birsa Agriculture University, Ranchi (Jharkhand) and Department of Genetics and Plant Breeding, I. Ag. Sc., BHU, Varanasi. List of winged bean genotypes used in the study are listed below in Table 1.

The research trial was conducted in a Randomized block design with three replications at Vegetable Research Farm in Banaras Hindu University, Varanasi, Uttar Pradesh during *kharif* season 2015-2016. The crop is grown on a field for the production of tubers; each genotype was sown in single row plots the seeds are normally sown 5-7.5 cm deep on ridges and earthed up after 15 to 20 cm seedling emergence. Each plot was consisted of one row of 5 m length with plant to plant and row to row distance being 50cm × 75cm, respectively. In each genotype per replication wise five plants randomly get to vegetative pruning (main branch and subsequent branch remove the shoot tip when plants attain height up to 1m) and reproductive pruning (from each peduncle or cluster all the flowers bud are removed except one which pod initiation has been started) to raise a good tuber yield. Tuber harvesting was done, when the all plant parts dried up to around 80percent. The total sugar content (mg/g), dry Matter (percent) and crude protein (percent) in tuber were estimated by using Anthrone method (Sadasivam and Manickam 1992, 1996); Micro-Kjeldahl's and AOAC methods (AOAC1995; Sawhney and Singh 2000), respectively.

Table 1: Winged bean genotypes used under present investigation.

Sr. No.	Genotype	S. No.	Genotype
1.	Ambika WB-11-1	12.	EC-178309
2.	Ambika WB-11-2	13.	IC-26904
3.	Ambika WB-11-3	14.	IC-38683
4.	Ambika WB-13-1	15.	IC-178292
5.	Ambika -13-5	16.	RMDWB-1
6.	Ambika WB-13-6	17.	RWB-2
7.	Ambika -13-4	18.	RWB-13
8.	EC-13084	19.	RWB-15
9.	EC-15018	20.	RWB-16
10.	EC-27884	21.	BHW-1
11.	EC-178289	22.	AKWB-1

The pre and post-harvest observations based on thirteen quantitative traits such as days to flower initiation, days to 1^{st} pod initiation, chlorophyll content at flower initiation (SPAD unit), chlorophyll content at 1^{st} pod initiation (SPAD unit), Leaf Area Index (LAI) at 1^{st} pod initiation, mature pods length (cm), mature pods width (cm), seeds per pod, 100 seed weight (g), days to tuber harvesting, tuber yield per plant (g), tuber length (cm), tuber breadth (cm), and four qualitative traits such as tuber moisture (percent), tuber dry matter (percent), tuber crude protein (percent) and total sugar content in tuber (mg/g) were recorded in five pruned plants selected at random from each genotype in each replication. The replicated mean data on seventeen tuber yield contributing traits were utilized for analysis of variance to test the significance for every character as per method given by Panse and Sukhatme (1967). Replicated data were used for D^2 statistics to estimate the genetic diversity through methodology given by Mahalanobis (1936) and clustering of genotypes into different clusters was done as per procedure given by Rao (1952). Finally, D^2 statistical analysis done by using WINDOSTAT version 9.2 statistical package at INDOSTAT services Hyderabad (Tiwari *et al.,* 2016).

RESULTS AND DISCUSSION

Mean square due to genotypes were exhibited highly significant difference at P<0.01 level for all the seventeen traits under studied indicating that there is an inherent genetic difference among the twenty two winged bean genotypes. Almost Similar type results noticed by Mahto and Dua (2009); Kushwaha *et al.* (2013); Sriwichai *et al.* 2021 in winged bean genotypes.

Assessment of genetic diversity. Genetic divergence was studied based on Mahalanobis D^2 Statistics. To study genetic divergence in 22 genotypes of winged bean data were subjected to D^2 analysis. Analysis employed Tocher method so as to group the genotypes into different clusters.

Group constellations. The accessions were grouped into five clusters. Cluster IV was the largest with 9 genotypes, followed by cluster II consisted 6 genotypes, cluster I having 5 genotypes, and cluster III & Cluster V having 1 genotype (Table 2, Fig. 1), indicating the dispersion of genotypes under divergent clusters. Genotypes belonging to the same cluster would be less divergent hence selection of these genotypes for further improvement in the crop will not effective. Therefore, hybridization involving genetically diverse parents belonging to different distant clusters would provide an opportunity to bring together gene constellations of diverse origin. Singh *et al.* (2002) by using Metroglyph analysis found divergence among 18 pure line genotypes derived from indigenous and exotic mixed population for ten traits in winged bean reported the strains were grouped into eight clusters. Mahto and Dua (2009) reported genetic divergence in 30 indigenous and exotic collections of winged bean grouped into nine clusters. Kushwaha *et al.* (2013) by using Mahalanobis D^2 statistics also reported genetic divergence among 26 germplasm lines of winged bean grouped into six clusters.

Inter and intra cluster distances among clusters. Detail insight into the diversity may help to select desirable genotypes for utilizing in breeding programmes, according to the objectives. The maximum intra cluster distance (D^2-values) was found in cluster IV (216.80) followed by cluster II (175.60), cluster I (125.25), cluster III and cluster V (0.00). The highest inter cluster distance was found between clusters IV and V (519.48) which was followed by cluster II and IV (417.59), cluster III and V (380.04), cluster I and V (372.85) and cluster II and III (296.51) (Table 3, Fig. 2). Thus, crosses may be made between the genotypes of the cluster IV and V to obtain better segregants as these recorded highest inter cluster distance. Singh *et al*. (2002) by using Metroglyph analysis, reported intra cluster distances ranged from 0.00 to 55. 14 while maximum inter cluster distance (259.31) was found between cluster IV and VIII. Kushwaha *et al.* (2013) was also reported range of intra clusters distance from 70.24 to 213.25 while inter clusters distance range from 234.78 to 1283.39 in winged bean genotypes.

Table 2: Clustering of twenty two winged bean genotypes by Tocher method.

Clusters	**Number of Genotypes**	**Name of Genotypes**
Clusters I	5	BHW-1, AKWB-1, RWB-16, RWB-2, Ambika WB-11-1
Clusters II	6	EC-13084, IC-38683, EC-178289, Ambika WB-11-3, 9 EC-15018, IC-26904
Clusters III	1	EC-27884
Clusters IV	9	Ambika WB-11-2, RWB-15, IC-178292, Ambika WB-13-6, Ambika -13-5, Ambika -13-4, EC-178309, Ambika WB-13-1, RWB-13
Clusters V	1	RMDWB-1

Table 3: Inter and intra Cluster distance (D^2 values) among twenty two genotypes of winged bean by Tocher Method.

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Cluster I	**125.24**	264.61	188.82	233.53	372.84
Cluster II		**175.59**	296.50	417.59	264.88
Cluster III			**0.00**	218.39	380.03
Cluster IV				**216.80**	519.48
Cluster V					**0.00**

In the present set of accessions, based on the inter-cluster distances suitable accessions may be selected for undertaking a suitable hybridization programme. It is observed that the germplasm desirable for different traits belong to different clusters. Therefore, selection of diverse accessions with desirable traits, and utilizing them in multiple crossing programmes, is expected to be effective in accumulation of desirable genes in to the common genetic background. Thus, an improved germplasm can be developed by utilizing such character specific diverse accessions in winged bean.

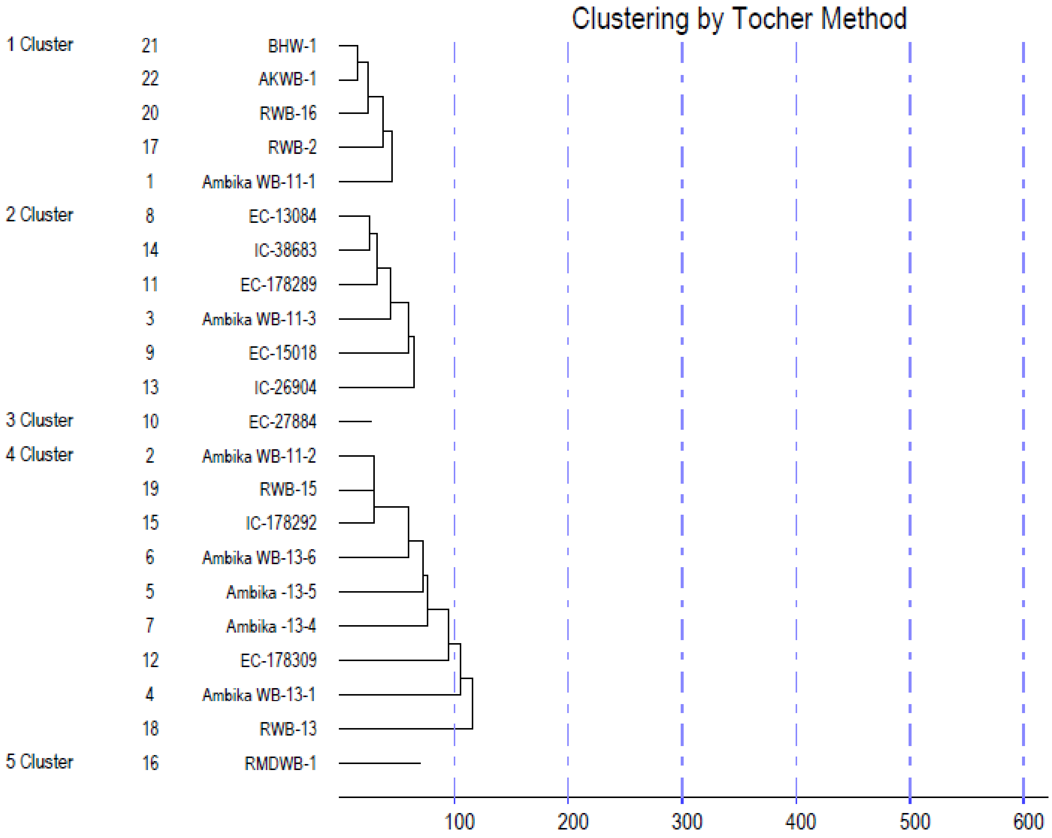

Fig. 1. The dendrogram clustering of twenty two genotypes of Winged bean by Tocher method.

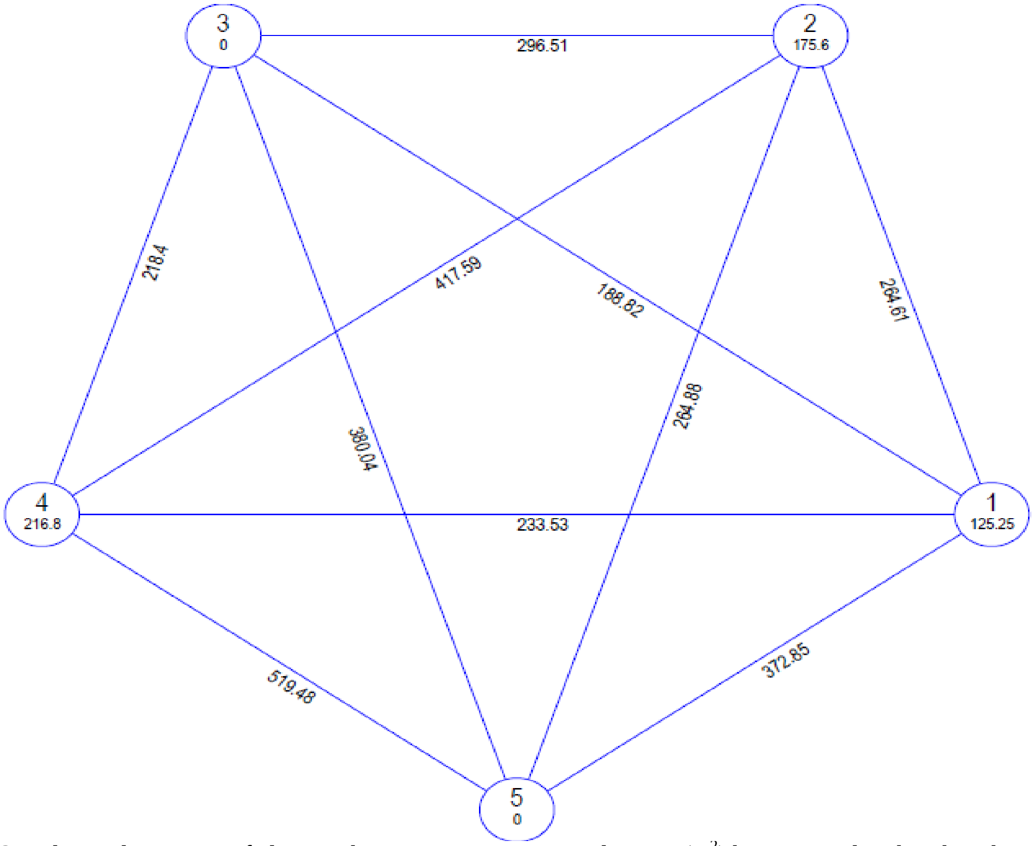

Fig. 2. Relative disposition of clusters showing average genetic distance (D^2) between and within them by Tocher method.

Performance of different traits in clusters. The inter cluster means of different traits exhibited significant variation between the clusters (Table 4): days to flower initiation, chlorophyll at flower initiation, LAI at 1st pod initiation and mature pods width in cluster III; days to pod initiation, days to tuber harvesting, tuber yield per plant, tuber length, tuber breadth, tuber dry matter and total sugar content in cluster V; chlorophyll at 1st pod initiation, LAI at 1st pod initiation, mature pods length, seeds per pod, 100 seed weight in cluster IV; tuber moisture in cluster II; and tuber crude protein in cluster I exhibited maximum values while comparing between the clusters. In this way their clusters exhibited maximum value for these characters across the clusters and therefore may contribute toward the hybridization programme initiated for the improvement of these characters.

Contribution of different characters towards divergence. The relative ranking of different character components of D^2 has been shown in the Table 5 which revealed that in all combinations of genotypes under study, the character total sugar content in tuber (mg/g) showed highest mean difference and therefore ranked first (82 times) on the basis of d_i values in each genotype and hence showed highest contribution percent is 35.50 followed by LAI at 1st pod initiation is 15.15, tuber breadth is 12.99,seeds per pod is 10.82, tuber dry matter is 8.23, mature pods width is 6.06, total crude protein is 4.76, chlorophyll at flower initiation is 4.33, chlorophyll at 1st pod initiation is 1.30, tuber length is 0.87 to the total divergence while days to flower initiation, day to 1st pod initiation, mature pod length, 100 seed weight, days to tuber harvesting, tuber yield per plant had no contribution towards divergence. Therefore, relatively simply inherited traits like total sugar content, tuber breadth, tuber crude protein, tuber dry matter, should be considered while choosing the parents from genetically diverse clusters. Kushwaha *et al*. (2013) was observed similar finding is that protein and sugars contributed maximum towards divergence among winged bean genotypes.

Table 4: Intra cluster means (Tocher method) for seventeen different traits in winged bean.

Characters Clusters	Days to flower initiation	Days to 1st pod initiation	Chlorophyll at flower initiation (SPAD Unit)	Chlorophyll at 1st Pod Initiation (SPAD Unit)	LAI at 1st Pod initiation stage	Mature Pods Length (cm)	Mature Pods Width (cm)	Seeds/ Pod	100 Seed weight (g)	Days to tuber harvesting	Tuber Yield per Plant (g)	Tuber Length (cm)	Tuber Breadth (cm)	Tuber Moisture (percent)	Tuber Dry Matter (percent)	Tuber Crude Protein (percent)	Total sugar content (mg/g) in Tuber
Cluster I	78.78	87.36	42.00	46.46	1.41	15.05	1.63*	9.58	24.51	145.17*	23.10	16.43	1.16	69.87	29.46	6.52**	340.12*
Cluster II	75.68	85.64	40.09*	45.69*	1.11*	13.86*	1.68	8.04	26.40	148.65	23.76	15.31	1.14	73.25**	26.03*	6.51	390.96
Cluster III	80.00**	86.24	43.60**	46.98	1.17	15.37	2.15**	10.70	24.66	150.30	16.21*	14.61*	0.99*	69.56	30.04	6.16	354.66
Cluster IV	71.92*	79.61*	42.06	47.22**	1.54**	16.31**	1.85	11.80**	26.98**	150.84	27.78	15.81	1.09	71.95	27.35	5.91	383.77
Cluster V	79.39	93.63**	42.07	45.71	1.13	14.14	1.70	7.30*	18.19*	150.87**	35.54**	18.37**	1.27**	65.33*	34.03**	5.36*	490.01**

**Highest values, *Lowest value

Table 5: Percent contribution of the individual traits towards total divergence in twenty two winged bean genotypes.

Source	Times Ranked 1st	Contribution percent
1. Days to Flower initiation	0	0.00
2. Days to 1st pod initiation	0	0.00
3. Chlorophyll at Flower initiation	10	4.33
4. Chlorophyll at 1st Pod Initiation	3	1.30
5. LAI at 1st Pod initiation	35	15.15
6. Mature Pods Length (cm)	0	0.00
7. Mature Pods Width (cm)	14	6.06
8. Seeds/ Pod	25	10.82
9. 100 Seed weight (g)	0	0.00
10. Days to tuber harvesting	0	0.00
11. Tuber Yield per Plant (g)	0	0.00
12. Tuber Length (cm)	2	0.87
13. Tuber Breadth (cm)	30	12.99
14. Tuber Moisture (percent)	0	0.00
15. Tuber Dry Matter (percent)	19	8.23
16. Tuber Crude Protein (percent)	11	4.76
17. Total sugar content (mg/g)	82	35.50

CONCLUSION

Analysis of variance exhibited highly significant differences among the genotypes for all seventeen traits under study. This studied indicate there is ample scope for selection of promising genotypes from present set of germplasms for tuber yield improvement. Mahalanobis's D^2 statistics was carried out in order to assess the genetic divergence among all the genotypes. Based on the inter-se genetic distance, twenty two winged bean genotypes could be grouped into five different clusters. Cluster IV, II and I contains 9, 6 and 5 accessions respectively, while clusters III and V contained only one genotype, indicating their distinctness among themselves and also from other genotypes under investigation. The intracluster distance was highest is 216.80 in cluster IV followed by cluster II and they also exhibited 2nd best inter cluster distance is 417.59 between them. The highest intercluster distance is 519.48 was found between cluster IV and V followed by cluster II and IV (417.59). On the basis of per se performance, Cluster V exhibited maximum mean value for days to 1st pod initiation, days to tuber harvesting, tuber length, tuber breadth, tuber dry matter, total sugar content in tuber, tuber yield per plant. Hybridization among the genotypes belonging to clusters IV (Ambika WB-11-2, RWB-15, IC-178292, Ambika WB-13-6, Ambika-13-5, Ambika-13-4, EC-178309, Ambika WB-13-1 and RWB-13) and V (RMDWB-1) was suggested to obtaining transgressive/ desirable segregants for developing high yielding cultivars with better quality in winged bean. The relative contribution of tuber traits to total divergence in this study indicated that total sugar content in tuber, tuber breadth, tuber dry matter percent and tuber crude protein percent had highest contribution towards total divergence. Therefore, selection of genotypes would be more effective based on the above traits.

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Conflict of interest. None.

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