Evaluation of Genetic Divergence in Spanish Bunch Groundnut (*Arachis hypogaea* Linn.) Genotypes

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ABSTRACT In our present study, we report the nature and degree of genetic diversity among 21 promising Spanish bunch groundnut genotypes of different agro-climatic regions, obtained from Directorate of Groundnut Research, Junagadh, Gujarat (India) during Kharif for 2015-16. The results obtained from analysis of variance showed that there was significant difference ($P < 0.05$) between the genotypes in terms of days to first flowering, days to maturity, 100-kernels weight, shelling percentage, sound matured kernel, and pod yield. The recorded data matrix on seven morphological traits were employed for Non-hierarchical Euclidean cluster analysis based on square Euclidian distance by Un-weighted Paired Group Arithmetic Average method. The 21 genotypes were grouped into four distinct non-overlapping clusters in a random sequence, indicating the presence of high dimension of genetic diversity. Pod yield as an economically important trait was found to be the key contributor towards the genetic divergence followed by days to maturity. Based on magnitude of divergence and performance towards yield and yield attributes, five genotypes (TG-75, AK-343, ICGV-07038, Girnar-3 and TCGS-1157) were selected for exploitation as parents in future breeding programs. Based on high yielding genotypes and large inter-cluster distances, a trial on crossing of the genotypes from cluster II with the genotypes of clusters IV and III, genotypes from cluster I with II emphasizing on selected five genotypes could result in wide spectrum of promising genetic variability, aiming at enhancement of groundnut yield.

Keywords Analysis of variance, Clustering, Mahalanobis D^2 , Pod yield, Squared Euclidian distance

INTRODUCTION

Cultivated groundnut (*Arachis hypogaea* Linn.) is the predominant one out of 69 species described in the genus *Arachis*. It's a self-pollinated, annual, herbaceous legume that belongs to the family Fabaceae. This crop, evolved in Southern Bolivia to North-Western Argentina regions of South America (Wiess 2000), is widely grown under wet conditions in semi-arid regions of > 80 countries including

Africa, Americas and Asia (Singh and Singh 1992) and have recourse both to human consumption as well as an important provenance of vegetative oil. The groundnut kernel serves a rich source of edible oil (48-50%) as well as protein (25-28%) (Janila *et al.* 2013). It is also a dietary source of vitamin E, niacin, falcin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium (Stigter *et al.* 2006) that are ingested right away as fresh, baked or cooked kernels otherwise, oil isolated from the

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kernel is consumed as cooking oil. The haulms (vegetative portions of plant) of groundnut deliver superior quality hay as animal feed since these are enrich with protein and ensure enhanced deliciousness as well as assimilation rate in comparison to other forage (Singh *et al.* 2010a). Groundnut now ranks fifth and thirteenth in the world among oil producing and food crops, respectively (Mondal *et al.* 2009). World production of groundnut is increasing and has reached 43,915,365 tons (in the shell). In India, groundnut is grown on 4,685,000 ha with a production of 6,557,000 tons and an average productivity of 13,996 hg/ha (FAOSTAT, <http://www.fao.org/faostat/en/#data/QC>).

Cultivated groundnut is an allotetraploid (AABB, $2n = 4x = 40$) having very narrow genetic diversity. Contrastingly, the diploid wild-type ancestors are inherently heterogeneous and preferably selected for cumulating stress resistant genes during adaptation to severe environments (Kochert *et al.* 1996; Subramania *et al.* 2000; Gimenes *et al.* 2002). The minimal level of variation in cultivated peanut has been accredited to three reasons or to combinations of those: (1) obstructions to gene flow from associated diploid species to domesticated peanut as a result of the polyploidization issue (Young *et al.* 1996); (2) fresh polyploidization, from one or a small number of individual(s) of each diploid parental species, combined with self-pollination (Halward *et al.* 1991); or (3) utilization of few leading breeding lines and few exotic germplasms in breeding programs, ensuing in a limited genetic base (Isleib and Wynne 1992). On the other hand, selection of genetically diverse parents, which is expected to develop superior and desirable segregants following crossing, enables the expansion of genetic base and development of superior genotypes (Gantait and Das 2009; Gantait *et al.* 2009). Majority of the economically important characters including pod yield and its components are amenable for genetic improvement through intense breeding among genetically diverse parents.

Keeping in view of the above genotypic aspects, the present investigation was carried out to know the nature and magnitude of genetic divergence in 21 groundnut genotypes for their incorporation in future hybridization program that would eventually help in development of groundnut varieties with higher pod yield.

MATERIALS AND METHODS

Plant materials and site of experiment

The Directorate of Groundnut Research (DGR), Gujarat, India, collected a total of 21 Spanish bunch-type promising genotypes from different agro-climatic zones/research centers of India. We've obtained these genotypes from the DGR (the details of the genotype collection sites have been updated in Table 1) and used as the experimental materials for the current analysis. The randomized block design was followed in the current trial at the In-Check C-block Experimental Farm (22°59'22.1"N 88°27'16.4"E), Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal situated at New Alluvial Zone of India, during Kharif (July to October for consecutive two years *i.e.* 2015-2016) season under non- irrigated condition. In each replication every genotypes was sown in four rows of 5 m length and 1.5 m width with a spacing of 30 cm between the rows and 10 cm between the plants within rows. All the advocated agronomic cultural practices and plant protection measures

Table 1. Summary of 21 Spanish bunch groundnut (*Arachis hypogaea* Linn.) genotypes used in the present experiment.

Genotype	Breeding center	State in India
AK-335	Akola	Maharashtra
CTMG-11	Chintamani	Karnataka
KDG-197	Digraj	Maharashtra
JCG-3005	Jagtial	Telangana/Andrapradesh
AK-343	Akola	Maharashtra
Dh-235	Dharwad	Karnataka
J-86	Junagadh	Gujarat
Dh-241	Dharwad	Karnataka
JCG-2141	Jagtial	Telangana/Andrapradesh
ICGV-07038	ICRISAT	Telangana/Andrapradesh
ICGV-06138	ICRISAT	Telangana/Andrapradesh
TG-74	Trombay	Maharashtra
TVG-0856	Vridhachalam	Tamil Nadu
TG-75	Trombay	Maharashtra
LGN-163	Latur	Maharashtra
VG-315	Vridhachalam	Tamil Nadu
TCGS-1157	Tindivanam	Tamil Nadu
OG-52-1	Bhubaneswar	Orissa
Girnar-3	Junagadh	Gujarat
TAG-24	Trombay	Maharashtra
R-2001-2	Raichur	Karnataka

were practiced whenever required in raising the crop. Hand weeding was carried out following 15 days of sowing and successively at 20 days interims to warrant nominal crop-weeds competition. There was no usage of chemical fertilizers, herbicides or pesticides.

Collection of data

Morphological data and yield data were recorded from every genotype. Ten randomly selected plants from respective row (*i.e.* 40 plants in each plot) of each genotype per replication for seven characters *viz.*, days to first flowering, days to 50% flowering, days to maturity, 100-kernels weight (g), shelling percentage, sound matured kernel (SMK) (%), pod yield (kg/ha) were sampled and their mean values were used for the statistical analysis. Nevertheless, days to first flowering, days to 50%

flowering and days to maturity were documented on the plot basis.

Statistical analysis

Collected data (replication-wise) for respective attributes were put through analysis of variance (ANOVA) using the SPSS (Version 11, SPSS Inc. Chicago, USA) software package. The collected data were assessed based on the Duncan's multiple range test (DMRT) (Duncan 1955) at a certain *P* value of 0.05 using SPSS. Using arc sine, percentage values were changed before calculating ANOVA and then were altered back to the primary scale (Compton 1994). Wilk's criteria were considered to estimate the cumulative influences of attributes towards variations among the genotypes. Subsequently, the Mahalanobis D^2 (Mahalanobis 1936) statistics was referred

Table 2. Mean values²⁾ of yield and yield contributing characters in 21 Spanish bunch groundnut (*Arachis hypogaea* Linn.) genotypes.

Genotype	First flowering (days)	50% flowering (days)	Days to maturity	100-KW (g)	Shelling (%) ³⁾	SMK (%) ³⁾	Pod yield (kg/ha)
AK-335	24.3d ^{y)}	32.5a	112.8def	40.9fg	67.9d-g	87.0cde	2855.5ab
CTMG-11	25.0bcd	32.3a	116.0a-d	38.5h	70.1a-f	89.3abc	1825.8fg
KDG-197	25.5bcd	32.5a	119.3a	44.7a-d	69.4a-g	86.0de	2774.0ab
JCG-3005	24.5cd	32.0a	114.3cde	39.4gh	67.0g	87.5b-e	1625.0g
AK-343	29.3a	33.8a	116.3a-d	40.8fg	70.9abc	90.8a	2604.8bc
Dh-235	27.3ab	33.5a	115.8a-d	38.3h	69.8a-g	86.0de	2131.5ef
J-86	24.5cd	32.0a	115.3b-e	45.8ab	68.9a-g	89.0a-d	2541.8bc
Dh-241	25.3bcd	31.8a	110.3fg	42.5def	67.3fg	87.0cde	2534.5bc
JCG-2141	27.0b	32.3a	114.8cde	42.4def	68.1c-g	89.5abc	2318.8de
ICGV-07038	24.3d	31.5a	118.5ab	39.2gh	68.2b-g	88.5a-e	2340.8de
ICGV-06138	26.0bcd	31.8a	116.3a-d	41.4efg	67.5efg	87.0cde	2785.0ab
TG-74	25.8bcd	31.3a	112.0ef	45.5abc	71.0abc	89.3abc	3080.5b
TVG-0856	25.5bcd	31.5a	116.8abc	43.3cde	68.9a-g	88.0a-e	2769.8ab
TG-75	26.3bcd	31.3a	114.0cde	44.7a-d	71.5a	87.0cde	3757.0a
LGN-163	25.5bcd	31.3a	116.5abc	44.2a-d	69.2a-g	85.5e	2559.0bc
VG-315	25.3bcd	32.0a	115.3b-e	44.3a-d	70.8a-d	90.3ab	2059.5ef
TCGS-1157	24.5cd	33.5a	116.0a-d	46.5a	71.5a	87.8b-e	3686.5a
OG-52-1	24.5cd	32.5a	108.0gh	43.6b-e	71.2ab	87.8b-e	2763.0ab
Girnar-3	25.8bcd	31.8a	106.3h	44.5a-d	70.6a-d	88.5a-e	3480.3a
TAG-24	26.8bc	31.8a	114.0cde	44.6a-d	71.2ab	90.0abc	2785.3ab
R-2001-2	26.8bc	31.5a	116.3a-d	44.0bcd	70.4a-e	88.3a-e	3592.5a

²⁾Data represent mean of four replicates with 40 samples per genotype.

^{y)}Data for each column followed by the different letters are significantly different according to Duncan's multiple range test (Duncan 1955) at the 0.05 probability level.

³⁾Data expressed as percentage were transformed using arc sine prior to ANOVA and converted back to the original scale for demonstration in the table (Compton 1994).

for the assessment of genetic diversity. Grouping of the genotypes was performed following the Tocher's method (Rao 1952) considering that the genotypes within the cluster have lesser D^2 values amid themselves in comparison to those from distinctive clusters. Squared Euclidian distance between genotypes was calculated from the standardized data matrix by Unweighted Pair Group Method using Arithmetic Averages (UPGMA) method, subsequently, the genotypes were grouped into different clusters using Statistical Analysis System (SAS).

RESULTS

Mean performance

The summary statistics of the morphological characters of all the genotypes (explained in Table 2) signified broad variation in respect to all the characters under consideration. The ANOVA computed for seven agro-morphological traits revealed the presence of significant differences ($P = 0.05$) on days to first flowering, days to maturity, 100-kernels weight, shelling percentage, SMK, pod yield. However, non-significant difference was observed on days to 50% flowering (Table 2). The mean performance of groundnut genotypes showed narrow range of means for studied agro-morphological traits except for pod yield, the most economically important trait (Table 2). In this study genotypes showed narrow range of variation for majority of the attributes, for instance, shelling percentage ranged from 67.0 to 71.5 with a mean of 69.6%, SMK ranged from 85.5 to 90.8 with a mean of 88.0%, first flowering ranged from 24.3 to 29.3 with a mean of 25.7 days, 50% flowering ranged from 31.3 to 33.8 with a mean of 32.1 days and 100-kernels weight ranged from 38.3-46.5 with a mean of 42.8 g. On the other hand, a wide range of

mean was recorded for pod yield between 1,625-3,757 with a mean of 2,708.1 kg/ha.

Cluster composition

In the present study, based on the D^2 values 21 genotypes were grouped into four distinctive non-hierarchical clusters. The components of these four clusters plus their origin are described in Table 3. Cluster analysis involves three key steps, *i.e.* computing the distances, associating the clusters then selecting the accurate figure of clusters. Out of the four clusters in our study, cluster II was the largest one containing 14 genotypes, cluster I having 3 genotypes, cluster III and IV were comprised of 2 genotypes each (Table 3). A dendrogram was also obtained from the non-hierarchical cluster analysis based on Euclidean's method that grouped the groundnut genotypes into four distinct non-overlapping clusters at a distance of 400 (Fig. 1).

Inter- and intra-cluster distances ($D = \sqrt{D^2}$)

Mean values of intra as well as inter-cluster distances among the genotypes revealed that the individual clusters possessed high values of intra-cluster distances. The highest intra-cluster distance was observed in cluster IV (5.822) (comprising TCGS-1157 and TAG-24) whereas the lowest distance value was recorded in cluster III (3.316) (comprising OG-52-1 and Girnar-3) (Table 4). The most plausible explanation for relatively short genetic distances amid OG-52-1 and Girnar-3 genotypes is that they might have descended from a mutual ancestral population. According to Singh *et al.* (2010b), the genotypes, inside individual cluster, having greater degree of divergence would generate more appropriate breeding materials for attaining the highest genetic advance. The genotypes TCGS-1157 and TAG-24 recorded the highest distance

Table 3. Clustering pattern of 21 Spanish bunch groundnut (*Arachis hypogaea* Linn.) genotypes.

Cluster No.	I	II	III	IV
No. of population	3	14	2	2
Names of genotypes	AK-335, TG-75, R-2001-2	CTMG-11, KDG-197, JCG-3005, AK-343, Dh-235, J-86, Dh-241, JCG-2141, ICGV-07038, ICGV-06138, TG-74, TVG-0856, LGN-163, VG-315	OG-52-1, Girnar-3	TCGS-1157, TAG-24

Fig. 1. Dendrogram representing classification/clustering of 21 Spanish bunch groundnut (*Arachis hypogaea* Linn.) genotypes on seven different quantitative characters based on Squared Euclidian distance matrix.

Table 4. Intra- (diagonal) and inter-cluster average distance for seven quantitative characters of 21 Spanish bunch groundnut (*Arachis hypogaea* Linn.) genotypes.

Cluster No.	I	II	III	IV
I	4.294	5.800	5.070	4.339
II		4.745	6.148	6.219
III			3.316	5.470
IV				5.822

within the same cluster (IV). However, the inter-cluster distances were higher than that of the intra cluster ones with an exception in case of cluster IV (Table 4). Such higher inter cluster distances indicates wide extent of genetic diversity among the genotypes of cluster I, II and III in particular. Another notably higher inter-cluster distance (6.148) was observed between cluster II and cluster III. The clusters that are isolated by the maximum statistical

distance display the greatest divergence for variability. Interestingly, the least distance (4.339) was displayed between cluster I and cluster IV.

Cluster means for various characters

Incomprehensible differences were recorded in the intra-cluster group means for six attributes out of seven (Table 5). Both cluster I (comprising 3 genotypes) and

Table 5. Cluster means values of seven different quantitative characters of 21 Spanish bunch groundnut (*Arachis hypogaea* Linn.) genotypes.

Cluster	First flowering (days)	50% flowering (days)	Days to maturity	100-kernel weight (g)	Shelling (%)	SMK ²⁾ (%)	Pod yield (kg/ha)
I	25.8	31.8	114.3	43.2	56.8	69.3	3401.7
II	25.8	32.1	115.5	42.1	56.3	69.9	2425.0
III	25.1	32.1	107.1	44.0	57.4	69.8	3121.6
IV	25.6	32.6	115.0	45.6	57.6	70.6	3235.9

²⁾SMK: stands for sound matured kernel.

cluster II (containing 14 genotypes) displayed highest cluster mean value (25.8) for days to first flowering whereas, cluster IV (with two genotypes) showed highest cluster mean of 32.6 for days to 50% flowering. In case of days to maturity, cluster II recorded the highest mean of 115.5. The superiority of cluster IV over the other three was also evident in case of 100-kernel weight (g) and SMK percentage, which were recorded as high as 45.6 and 70.6, respectively. The most significant differences among the cluster means were observed in case of pod yield wherein cluster I recorded maximum mean value of 3,401.7 kg/ha. Interestingly, cluster II comprising highest number of genotypes and displaying highest mean days to maturity recorded minimum mean value of pod yield (2,425 kg/ha). Cluster IV, having least number of genotypes but securing maximum mean value of 100-kernel weight (g) and SMK percentage, ranked second based on mean pod yield (3,235.9).

Contribution of various characteristics to total divergence

The percent contribution assay of all the seven traits concerning the influence of overall genetic divergence specified that pod yield was the highest contributor with 70.47% out of 100. Pod yield was followed by days to maturity (11.43%), SMK (%) (7.62%), 100-kernel weight (5.24%), shelling (%) (2.86%) and days to first flowering (2.38%) (Table 6). It was interesting to observe in our study that 50% flowering showed no contribution towards genetic diversity. It was also evident that pod yield alone, being the prime and economically most desirable trait, played the key role to establish genetic divergence among groundnut genotypes. The other five traits merely con-

Table 6. Percent contribution of each quantitative character to genetic divergence 21 Spanish bunch groundnut (*Arachis hypogaea* Linn.) genotypes.

Characters	No. of first rank	Contribution (in percent)
First flowering	5	2.38
50% flowering	0	0
Days to maturity	24	11.43
100-kernels weight (g)	11	5.24
Shelling (%)	6	2.86
SMK ²⁾ (%)	16	7.62
Pod yield (kg/ha)	148	70.47
Total	210	100

²⁾SMK: stands for sound matured kernel.

tributed 29.53% with days to maturity being the second major contributor of total divergence.

Promising genotypes from diverse clusters

The proficient genotypes belonging to diverse clusters were selected based on the mean performance for different traits for their exploitation as the parents in hybridization program. The selected genotypes are enlisted according to their merit for six different characters under study except days to 50% flowering (Table 7) since it was not a contributor during assessment of genetic divergence (Table 6). The promising genotypes are TG-75 (with high pod yield and shelling percentage) from cluster I, AK-343 (with high SMK %), and ICGV-07038 (with early flowering) from cluster II, Girnar-3 (with early maturity) from cluster III, and finally TCGS-1157 (with high 100-kernel weight, high shelling percentage and high pod yield) from cluster IV.

Table 7. Promising genotypes of Spanish bunch groundnut (*Arachis hypogaea* Linn.) selected from diverse clusters for different yield attributing characters.

Cluster no.	Genotype(s)	Trait(s)
I	TG-75	Pod yield (3757 kg/ha), Shelling (%) (71.5)
II	AK-343	SMK ²⁾ (%) (90.8)
	ICGV-07038	First flowering (24.3 days)
III	Girnar-3	Early maturity (106.3 days)
IV	TCGS-1157	100-KW ²⁾ (g) (46.6), Shelling (%) (71.5), Pod yield (kg/ha) (3686.5)

Note: 50% flowering was not considered since it was not significant contributor during assessment of genetic divergence.

²⁾SMK and KW stand for sound matured kernel and kernel weight, respectively.

DISCUSSION

In this current report, genetic diversity was studied amongst 21 groundnut genotypes based on seven quantitative attributes. The ANOVA outcome indicated the presence of variability among the groundnut genotypes on studied traits that can be exploited through selection to improve the crop for desired traits in further groundnut breeding program. The cumulative influences of all the seven attributes were examined by the Wilk's criteria that signified the variations among the genotypes. In the present study, grouping of the genotypes was performed following the Tocher's method (Rao 1952) considering that the genotypes within the cluster have lesser D^2 values amid themselves in comparison to those from distinctive clusters. The genotypes were randomly distributed in distinct clusters. The configuration of genotype distribution among four clusters established the presence of divergence amid the genotypes, which likewise was specified by the ANOVA. Nevertheless, the genotype distribution in four clusters was independent of high yield and its attributes, the key parameters for which the genotypes were screened. The probable reason for such dissimilarity among genotypes might be attributed to the divergent genetic background of the parents and different gene assemblies that govern the adaptive characters. This is in contrary to the earlier report of Vasanthi *et al.* (2015), which revealed that the distribution of genotypes into different clusters was related to the objective with which the genotypes were bred. Clustering pattern showed significant impact on selection of parents and the cross combinations aiming to create maximum possible variability for various characters.

For further selection and improvement, the genotypes having high divergence values selected from any cluster can be included in hybridization scheme (Raghuwanshi *et al.* 2015).

In our study, it was observed that cluster II and cluster IV are highly dissimilar so as cluster II and cluster III suggesting that the genotypes from these two groups could be assessed for their combining ability and could be used as parents in heterosis breeding programs in groundnut as suggested by Betrán *et al.* (2003) in maize and Mahmuda *et al.* (2008), in *Brassica*. Genotypes that are separated by more statistical distance can be selected from those inter-clusters for their utilization in hybridization program (Maity *et al.* 2015). This statement is corroborated by the report of Bhakal and Lal (2015) who suggested that crossing between the groundnut genotypes allied to the cluster pairs cleaved apart by higher inter-cluster distances might develop anticipated transgressive segregates that eventually opens up the opportunity for identification of superior genotypes in successive generations. Our results and subsequent recognition of divergent groundnut accessions having high pod yield could be suitable in breeding for genetic improvement. Additive gene effects will play a primary role in inheritance of quantitative traits if there are less divergent germplasm lines and adapted lines (Isleib and Wynne 1983). With the expansion of diversity between parents the dominance effects and epistatic variations start to play substantial roles in the inheritance of quantitative traits (Halward and Wynne 1991). In case of self-pollinated crop such as peanut, this would have inferences during determination of an apt parent selection scheme.

It further evidently appears from the above results that there was wide diversity from one cluster to the other in respect of cluster means for all seven traits especially pod yield, the most economically important attribute. This situation indicates that the genotypes that are markedly different in their mean performance for various characters were separated into different clusters. For the selection of desirable recombinants in the succeeding populations of groundnut a crossing scheme might be planned among the genotypes those belong to the cluster pairs with large inter-cluster distance and possessing high cluster means for single or multiple traits. Similar suggestions were also advocated by Gupta and Vachhani (2015) in Virginia type groundnut. According to Suneetha *et al.* (2012), the statistics of proportional contribution of different quantitative traits towards divergence assist the breeder for selection of parents for hybridization and in further advance generations. The efficacy of pod yield to be the major contributor in our study corroborates with the earlier reports on legumes. Therefore, it is logical that emphasis should be given on pod yield and days to maturity during selection of parents for hybridization *i.e.* selection of genotypes as divergent parents based on two traits may lead to broad spectrum of favourable genetic variability for yield improvement in groundnut (Gupta *et al.* 2015). Our results are in consistency with those of the previous reports on groundnut (Sumathi and Muralidharan 2008; Sonone and Thaware 2009; Sonone *et al.* 2011).

The variability parameters for yield and component traits in some of newly developed groundnut genotypes which have not been reported earlier by the researchers, will be helpful for harnessing present variability among them which in turn can support the ongoing and future groundnut breeding programs. It could be established that all the genotypes were observed to be divergent. Five highly divergent genotypes that showed significant performance for assorted characters under study have been identified and could be endorsed for crossing with other genotypes of the clusters exhibiting high inter-cluster distances. It's a well-known statistics that, more the genetically diverse parents employed in hybridization scheme, higher will be the probabilities of attaining heterotic hybrids and broad-spectrum variability in segregating

populations. Furthermore, there is a probability that the most productive hybrids might derive from high yielding parents having a high genetic diversity. Hence, in our study, based on high yielding genotypes and large inter-cluster distances, a trial on crossing of the genotypes from cluster II with the genotypes of clusters IV and III, genotypes from cluster I with II emphasizing on TG-75, AK-343, ICGV-07038, Girnar-3 and TCGS-1157 could be logical. The outcome might result in wide spectrum of promising genetic variability aiming at enhancement of groundnut yield.

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