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Diversity Study in Garlic for Nutritional Value and Bio-functionality

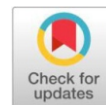
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ABSTRACT

Garlic (*Allium sativum* L.) is an important bulb vegetable. Sexual propagation in cultivated garlic is not feasible and this is the major challenge faced in garlic improvement programme. Improvement in garlic largely depends upon the selection of new variants followed by their asexual propagation. Garlic is regarded as a functional food and owes its antioxidant properties to these flavonoid and phenolic compounds. The phenolic and flavonoid contents in garlic vary to some extent with agronomic and environmental factors but genotype accounts for the primary factor that contributes towards this variation. Hence, twenty five genotypes of garlic were characterised for polyphenolic content and antioxidant properties and diversity analysis was worked out. The work was carried out in the Department of Horticulture (Vegetable and Floriculture), Bihar Agricultural University, Sabour. The experiment was laid out in randomized block design and recommended package of practice was followed for raising the crop. The results clearly indicated highly significant variations among genotypes of garlic which also showed marked divergence for all the biochemical parameters namely, TSS, ascorbic acid, total carotenoids, total phenolic content, total flavonoids and antioxidant capacity, RSA, MCA, nitrogen%, phosphorous%, potassium%, sulphur%. Cluster analysis of genotypes showed that all the genotypes were grouped into six clusters. Cluster I consisted a maximum number of genotypes and Cluster VI contained a minimum number of genotypes. The highest intra-cluster distance was recorded for cluster VI lowest intra-cluster distance was recorded for cluster V. The highest inter-cluster distance was observed between cluster V and indicating wider genetic diversity among the genotypes between these groups. Cluster VI had the highest mean value for the parameters like total phenol content (1097.53), antioxidant capacity (10.15), radical scavenging activities (48.64), Phosphorous% (0.61), and Sulphur % (8.39). The contribution towards divergence due to the biochemical parameters like Flavonoid content (19.67), Antioxidant activity (13), protein% (10), potassium% (9.33), nitrogen% (9), Phosphorous% (8.67), MCA (8.67) and RSA (7.67) were considerably high.

Keywords: Garlic, diversity, phenols, minerals, antioxidant and radical scavenging properties

Introduction

Garlic is an important bulb vegetable that is regarded as a functional food. It owes its antioxidant properties to the flavonoid and phenolic compounds present in it. However, not all the genotypes of this magic herb are equally potent. Therefore, it is important to estimate the genetic variation and assess genetic diversity for the management and utilization of germplasms.

Garlic (*Allium sativum* L.) belongs to the family Amaryllidaceae and is the second most widely used *Allium* next to onion. Garlic (*Allium sativum* L.) is a monocotyledonous vegetable that has its origin in Central Asia (Kazakhstan), with secondary centers of diversification in China and the Mediterranean area [1] (Etoh and Simon, 2002). Garlic is among the earliest domesticated plants and is cropped worldwide. There are different types or subspecies of garlic, and most notable are the hard neck garlic (*Allium sativum* var *ophioscordon*) and soft-neck garlic (*Allium sativum* var *sativum*) [2].

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The hard-necked garlics are the primitive garlics and the soft-necked are the ones that have been developed or cultivated over the centuries from the hard-neck garlic through a process of selection. Right kind of garlic has to be recommended for a given latitude, since it can be day-length sensitive. In general, hard neck garlic is generally grown in cool climates, while the soft-neck is grown closer to the equator [3].

Garlic is a diploid species ($2n = 2x = 16$) of obligated apomixes therefore its reproduction is vegetative [4]. Although some garlic plants found in the Campania region of Italy were shown to be tetraploid ($4n = 32$), while some cultivars might be triploid [5]. It has been reported that garlic is one of the oldest cultivated vegetables and its positive effect on human being has been known for thousands of years. It is grown throughout the world and used as spices and flavoring agent for many foods [6]. It is mostly used for culinary purposes and as a condiment for different food items. The garlic bulb is the most commonly used part of the plant. With the exception of the single clove types, garlic bulbs are normally divided into numerous fleshy sections called cloves. Garlic cloves are also used for consumption (raw or cooked) or for medicinal purposes. They have a characteristic pungent, spicy flavour that mellows and sweetens considerably with cooking. Other parts of the garlic plant are also edible. The leaves and flowers (bulbils) on the head are sometimes eaten.

They are milder in flavor than the bulbs, and are most often consumed while immature and still tender. Green stalks and young leaves also are eaten fresh or cooked and furthermore, large quantities of garlic used for pharmaceutical purposes [7]. As spices, it contains allicin, which gives odour of true garlic. A large number of sulphur compounds contribute to the smell and taste of garlic. Allicin has been found to be the compound most responsible for the "hot" sensation of raw garlic. Allicin, along with its decomposition products diallyl disulfide and diallyl trisulfide are major contributors to the characteristic odor of garlic. According to the traditional Indian medication - Ayurveda, it is used in treatment for diseases like running cold, saliva formation, asthma, influenza, diarrhoea, etc. Garlic cloves are used as a remedy for infections (especially chest problems), digestive disorders, and fungal infections such as thrush. Garlic can be used as a disinfectant because of its fungicidal and bactericidal properties [8]. It is a rich source of protein, phosphorus, calcium, magnesium, potash, and ascorbic acid. In one fresh peeled garlic cloves having 62.8% moisture, 6.3% protein, 0.1% fat, 0.8% fibre, 29.0% carbohydrates, 0.03% calcium and 0.31% phosphorus [9]. Considering the innumerable benefits of garlic on health, nutritional and medicinal front, it can be very well regarded as a functional food. Foods which yield health benefits apart from fundamental nutrition as a result of physiologically active food components are called functional foods [10]. By advancing the immune system and preventing diseases and degenerative disorders, functional foods produce physiological or metabolic advantages [11]. Functional foods are the natural and traditional foods commonly used for human consumption and such foods are often high in nutrition which decreases the risk of disease and enhances the physical and mental health benefits [12]. Garlic very rightly fits and fulfils the standards of functional food and this fact has also been documented [10]. For thousands of years, it has been used for a great many different medicinal purposes and its effects can be attributed to its diverse physiologically active organosulfur components [13]. It has been reported that organosulfur components of garlic employ numerous physiological effects, such as hypocholesterolemic activity, antithrombic effect, inhibition of platelet aggregation, antimicrobial activity, lipid-lowering effect, hypoglycemic activity, and lipoxygenase and tumor inhibition [14]. Garlic is now claimed as one of the important element in daily diet which also acts as a functional food. However, not all the genotypes of this magic herb can be equally potent with respect to organosulfur compounds are responsible for the functional properties in garlic. The principal bioactive compound called "Allicin" and many other nutrient and antioxidant properties of garlic varies with genotypes which shows great degree of diversity.

The knowledge about the genetic diversity of a crop species therefore is very important and prerequisite for its exploitation. Abundant garlic germplasm resources broaden the genetic variability and provide considerable opportunities for genetic research and breeding [15]. Earlier studies have shown that garlic contains huge genetic variation [16]. The intelligent exploitation of garlic accessions for genetic analyses requires a detailed knowledge of genetic diversity and historical relationships among the accessions [15]. A large scale diversity of different ecotypes has been established over time in various areas of cultivation According to incomplete statistics, there are more than 2000 varieties kept in different countries [17].

Characterization of germplasm collections based on their bulb concentration of phytochemicals and traits associated with garlic nutraceutical properties is important for the identification and selection of garlic clones with high functional value. This is particularly relevant in this species, for which conventional breeding strategies by means of sexual reproduction are not readily feasible and, therefore, its improvement largely relies on the identification and selection of new variants followed by their asexual propagation. Thus it is not only important to estimate the genetic variation amongst the gemplasms for selection of diverse parents which may be useful as donors to complement various breeding methods [18-19] but an adequate assessment of its genetic diversity is necessary for its management and utilization in breeding programs.

2. Materials and Methods

2.1 Experimental Framework

The experiment was conducted at Vegetable Research Farm, Department of Horticulture, Bihar Agricultural University, Sabour, Bhagalpur during the Rabi season. The experimental material consisted of 25 garlic genotypes from different locations in India (Table-1). Experimental trial was laid out in a Randomised Complete Block Design with three replications. Cloves of each genotype were sown at a distance of 15 cm from the row to row and 10 cm from plant to plant. A standard package of practices to raise a successful crop was followed. Observations were recorded on five randomly selected plants from each genotype in every replication. The study was done for important biochemical parameters like total soluble solids, ascorbic acid, total phenols, total carotenoids, total flavonoids and biological functions like radical scavenging activity and metal chelating activity. The technique of analysis of variance for Randomised Block Design was adopted, as suggested by Panse and Sukhatme [20]. The D^2 statistics for a measure of group distance was worked out as has been given by P. C. Mahalanobis [21]

2.2. Biochemical content and biological functions analysis

Three biological replicates for each garlic accession, consisting each replicate of 5 cloves obtained from 5 different bulbs (one clove per bulb), were used for analysis of ascorbic acid, total phenolics, total solids content, total carotenoids, total flavonoids content, Nitrogen, Phosphorus, Potassium and Sulphur, Radical scavenging activity, antioxidant capacity, Metal chelating activity. Thus, in total, 15 garlic bulbs were sampled per accession per analysis. The digital refractometer was used for the measurement of total soluble solids. Ascorbic acid content of the garlic was determined by titrating freshly extracted juice against 2, 6-dichlorophenol indophenols dye [22]. Total phenolic content of garlic bulb was determined by the method laid down by Singleton et al. [23] using a Dynamica, Australia, UV spectrophotometer. The reagent used was Folin-ciocalteau and the absorbance was measured at 735 nm. Total carotenoids content in garlic bulb was determined by the method of Roy [24] with some modifications. The sample absorbance was measured at 452 nm in a Dynamica, Australia, UV spectrophotometer, using petroleum ether as blank. The radical scavenging activity (RSA) was estimated using the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay [25]. The chelating effect was determined according to the method of Dinis et al. [26]. Absorbance of the solution was measured by using an UV spectrophotometer at 562 nm.

The cupric ion-reducing antioxidant capacity was determined according to the method of Apak et al. [27]. The absorbance was recorded at 450 nm against the reagent blank. Total nitrogen content in the samples was estimated through digestion followed by distillation by Kel-Plus unit using the method of Nelson and Sommers [28]. Phosphorus content in samples was determined by vanado molybdophosphoric yellow colour method. Diacid mixture of nitric acid and perchloric acid was used for the digestion of bulbs. Potassium content in plant samples was determined by flame photometry [29]. Diacid mixture of nitric acid and perchloric acid was used for the digestion of bulbs. Sulfur content in plant was determined by the turbidimetric method [30]. Diacid mixture of nitric acid and perchloric acid (in ratio of 10:4 v/v) was used for the digestion of bulbs. Transmittance was measured by using a spectrophotometer at 420 nm

3. Results and Discussion

3.1 Results

The analysis of variance (Table-2) indicated highly significant variations among genotypes of garlic which also showed marked divergence for all the biochemical parameters namely, TSS, ascorbic acid, total carotenoids, total phenolic content, total flavonoids and antioxidant capacity, RSA, MCA, nitrogen%, phosphorous%, potassium%, sulphur%.

Cluster analysis of genotypes was performed and on the basis of Mahalanobis D² values, all the genotypes were grouped into six clusters (Fig.-1). Among the different clusters, cluster I consisted maximum number of genotypes (9 genotypes) followed by cluster II and III (4 genotypes), cluster IV and V (3 genotypes) and VI contained (2 genotypes) contained minimum number of genotypes. The clustering pattern clearly reflects the presence of considerable extent of genetic diversity in the material under study (Table-3). The highest intra-cluster distance was recorded for cluster VI (1333.953) followed by cluster III (790.881). Lowest intra-cluster distance was recorded for cluster V (454.323). Other clusters have moderate intra-cluster distances like cluster IV (724.16), cluster I (640.639) and cluster II (540.741). The inter-cluster distance ranged from 849.909 (between cluster I and II) to 2585.076 (between cluster V and VI). The highest inter-cluster distance was observed between cluster V and VI (2585.076) followed by clusters I and VI (2229.119), cluster IV and VI (1969.558), cluster II and VI (1899.802) and cluster III and VI (1562.167) indicating wider genetic diversity among the genotypes between these groups. The mean value of six different clusters for the biochemical parameters under study presented in Table-3 clearly indicates that Cluster III showed lowest mean value for Nitrogen% (0.56), Phosphorous% (0.36) and Protein% (3.56). However, Cluster VI had the highest mean value for the parameters like total phenol content (1097.53), antioxidant capacity (10.15), radical scavenging activities (48.64), Phosphorous% (0.61), and Sulphur % (8.39). Different traits have different degree of contribution towards diversity of the genotypes. The percentage contribution of each character towards total genetic divergence has been presented in the Table-4 & Fig.-2. The contribution towards divergence due to the biochemical parameters like Flavonoid content (19.67), Antioxidant activity (13), protein% (10), potassium% (9.33), nitrogen% (9), Phosphorous% (8.67), MCA (8.67) and RSA (7.67) were considerably high.

3.2. Discussion

The exploration of genetic diversity in the available germplasm is a prerequisite in a breeding programme for the effective selection of superior genotypes. In the process of genetic improvement of any crop, genetic diversity among germplasm plays a major role, since it opens the way to determine the most divergent parents based on the contribution of different qualitative and quantitative traits, for further utilization in any hybridization programme. A plant breeder, therefore, sets hopes of improvement on the extent of genetic variation and degree of improvement possible on the beneficial genetic variability [31]. For selecting right parents for breeding the knowledge of genetic divergence is essential. Therefore divergence study was carried out for 25 garlic genotypes and it was observed that the genotypes differed significantly with regard to the biochemical characters and showed marked divergence. Out of several methods available, Mahalanobis generalized distance estimated by D² statistic [32] is a unique tool for discriminating populations considering a set of parameters together rather than inferring from indices based on morphological similarities and phylogenetic relationships. On the basis of Mahalanobis D² values all the genotypes under present study were grouped into six clusters. Among the different clusters, cluster I consisted maximum number of genotypes while cluster VI contained minimum number of genotypes. These results are in conformity with the findings Singh et al [33] and Sandhu et al. [19]. The grouping pattern of the genotypes suggested no parallelisms between genetic divergence and geographical distribution of genotypes, Singh and Dubey [34] and Mohanty & Prusti [35] also reported that genotype diversity was independent of geographical region. The inter-cluster distance was greater than intra-cluster distance in among the genotypes under study, which revealed that there was greater divergence among genotypes between clusters than within clusters. The highest intra-cluster distance was recorded for cluster VI followed by cluster III, cluster IV, cluster II and cluster V. Hence, selection within genotypes in these clusters may be exercised based on mean value for the traits of interest. The highest inter-cluster distance was observed between cluster V and VI followed by cluster I and VI, cluster IV and VI, cluster II and VI and cluster III and VI, indicating wider genetic diversity in the genotypes between these groups. The lowest inter-cluster distance between cluster III and IV, indicating closer relationship and similarity for most of the traits among various genotypes in these clusters. Similar types of findings were reported by Jabbes et al. [36], Singh et al. [37] and Sandhu et al. [19]. However, for obtaining favourable broad spectrum genetic variability for yield and quality, selection of genotypes based upon greater cluster distance may be made. Genotypes belonging to the cluster with maximum inter cluster distance were genetically more divergent [37]. Putative parents for any crop improvement programme belonging to different clusters are characterized by large inter-cluster distances and thus, may be used for crop improvement programme.

Estimates of the cluster means for different traits are measures of inter cluster divergence and degree of homogeneity. Hence, cluster means were worked out which indicated that different clusters were distinctly different with respect to various traits. Cluster VI had highest mean value for Total Phenolic content followed by Antioxidant activity, RSA, Phosphorous% and Sulphur %. However, Cluster III showed lowest mean value for Nitrogen% followed by Phosphorous% and Protein%.

Therefore genotypes grouped under cluster IV may be potent source for phenolic compound and antioxidant properties in garlic improvement programme for quality characters from medicinal point of view. High concentrations of several phenolic compounds, including flavonoids have also been reported by Beato et al. [38] and it is because of this that garlic has been proven to have anti HIV property and the ability to protect LDL cholesterol from oxidation, and lesser threat of cardiovascular diseases [39]. Garlic is known to be useful against diseases due to its scavenging nature for oxygen free radicals. Capacity of allicin which is the active component in garlic to scavenge the hydroxyl radicals is well recognized. The decline in hydroxyl radicals has revealed the effectiveness of the antioxidant property of allicin by impediment of lipid peroxidation [40]. Cluster IV also has the genotypes having high mean value for sulphur percentage. The anti microbial properties in garlic are due to the organosulfur compounds present in it. The organosulfur compounds of garlic such as allyl-sulfides can also checks the risk of hormone related cancers and chemically induce tumors [41]. The beneficial effect of garlic on diabetes mellitus is mainly attributed to the presence of volatile sulfur compounds, such as alliin, allicin, diallyl disulfide, diallyl trisulfide, diallyl sulfide, S-allyl cysteine, ajoene and allyl mercaptan. Garlic extracts have been reported to be effective in reducing insulin resistance [42]. Relative contribution of characters towards genetic divergence may be beneficial for selection in crop improvement. The individual characters contributing maximum to the D^2 values have greater emphasis for deciding the cluster for the purpose of further selection.

Flavonoid content contributed maximum towards total divergence, followed by antioxidant activity, protein%, potassium%, nitrogen %, phosphorous%, MCA and RSA garlic. The present findings were in line with the findings of Chen et al.[43].

4. Conclusion

In the present study garlic genotypes under study showed significant variations among and also showed marked divergence for all the biochemical parameters namely, TSS, ascorbic acid, total carotenoids, total phenolic content, total flavonoids and antioxidant capacity, RSA, MCA, nitrogen%, phosphorous%, potassium%, sulphur%. Cluster analysis of genotypes showed that all the genotypes were grouped into six clusters. Cluster I consisted maximum number of genotypes and cluster VI contained a minimum number of genotypes. Cluster VI had highest mean value for the parameters like total phenol content (1097.53), antioxidant capacity (10.15), radical scavenging activities (48.64), Phosphorous% (0.61) and Sulphur % (8.39). The contribution towards divergence due to the biochemical parameters like Flavonoid content (19.67), Antioxidant activity (13), protein% (10), potassium% (9.33), nitrogen% (9), Phosphorous% (8.67), MCA (8.67) and RSA (7.67) were considerably high. Therefore, the data generated from the present experiment might be useful to for the identification of the genetic diversity among genotypes which could be useful for marker-assisted selection for further crop improvement programmes in future.

Table 1: Genotypes of Garlic under investigation

	Genotypes	Source
1.	IC 344844	Collection from DOGR maintained at BAU, Sabour
2.	G 50	Collection from DOGR maintained at BAU, Sabour
3.	BRG-1	Local collection maintained at BAU, Sabour
4.	Phule Basant	Collection from DOGR maintained at BAU, Sabour
5.	WG 22	Collection from DOGR maintained at BAU, Sabour
6.	IC-337433	Collection from DOGR maintained at BAU, Sabour
7.	BRG-10	Local collection maintained at BAU, Sabour
8.	BRG-3	Local collection maintained at BAU, Sabour
9.	RG482	Collection from DOGR maintained at BAU, Sabour
10.	WG-323	Collection from DOGR maintained at BAU, Sabour
11.	IC-374951	Collection from DOGR maintained at BAU, Sabour
12.	RG 61	Collection from DOGR maintained at BAU, Sabour
13.	IC 37506	Collection from DOGR maintained at BAU, Sabour
14.	638	Collection from DOGR maintained at BAU, Sabour
15.	G282	Collection from DOGR maintained at BAU, Sabour
16.	IC 141151	Collection from DOGR maintained at BAU, Sabour
17.	IC 373010	Collection from DOGR maintained at BAU, Sabour
18.	IC-372974	Collection from DOGR maintained at BAU, Sabour
19.	G323	Collection from DOGR maintained at BAU, Sabour
20.	IC-15642	Collection from DOGR maintained at BAU, Sabour
21.	M-118	Collection from DOGR maintained at BAU, Sabour
22.	IC-375107	Collection from DOGR maintained at BAU, Sabour
23.	M-90	Collection from DOGR maintained at BAU, Sabour
24.	650	Collection from DOGR maintained at BAU, Sabour
25.	BRG-6	Local collection from maintained at BAU, Sabour

Table- 2: Analysis of variance for biochemical characters of garlic genotypes under study

Characters	Mean Sum of Square		
	Replication	Genotypes	Error
	df=2	df=24	df=48
Ascorbic acid	3.219	112.370**	3.639
Total phenolic content	211.234	119551.258**	3373.741
Total soluble solid	32.969	97.436**	3.453
Antioxidant capacity	0.055	4.701**	0.091
Total flavonoid	20.517	5485.91**	33.673
Radical scavenging activity	3.210	358.116**	4.934
Metal chelating activity	7.550	613.414**	12.591
Total carotenoid	1.621	57.068**	0.782
Nitrogen%	0.001	0.403**	0.007
Phosphorous%	0.002	0.041**	0.003
Potassium%	0.002	0.029**	0.002
Sulphur%	0.009	3.220**	0.145
Protein%	0.005	15.256**	0.119
*, ** are significant at 5% and 1% levels of significance respectively.			

Table-3: Clusters Based on 25 Genotypes of Garlic

Clusters	Genotypes
Cluster I	IC 344844, IC-37506, RG 61, IC 141151, IC-15642, BRG-10, IC-372974, RG482, BRG-6
Cluster II	M-90, 650, G282, IC-375107
Cluster III	IC-337433, G 323, IC 373010, IC-49387
Cluster IV	G50, Phule Basant, WG-323
Cluster V	BRG-1, M-118, BRG-3
Cluster VI	WG 22, 638

Table- 4: Average of intra (diagonal) and inter cluster distance

	Cluster	Cluster	Cluster	Cluster	Cluster	Cluster
	I	II	III	IV	V	VI
Cluster I	640.639	849.909	956.098	1177.938	1044.16	2229.119
Cluster II		540.741	1121.755	1047.741	1062.005	1899.802
Cluster III			790.881	1477.968	1424.561	1562.167
Cluster IV				724.16	1354.293	1969.558
Cluster V					454.323	2585.076
Cluster VI						1333.953

Table-5: Mean values of six clusters for the biochemical characters of garlic genotypes under study

Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Ascorbic Acid(mg/100g Fw)	25.10	31.05	31.38	21.46	23.49	26.86
Total phenolic content (mg GE/100g Fw)	785.59	708.97	896.71	665.30	616.39	1097.53
Total Soluble Solid(%)	38.44	33.79	38.66	39.30	32.44	28.23
Antioxidant activity (µmTE/g FW)	6.91	6.84	8.15	8.25	8.00	10.15
Flavonoid content (mg CE/100g FW)	121.49	97.68	111.18	127.84	100.90	171.70
RSA (%)	36.60	46.66	48.30	29.25	41.99	48.64
MCA(%)	71.97	58.42	70.63	33.66	70.93	65.66
Total carotenoid(mg/100g FW)	14.67	11.26	16.02	12.30	19.82	11.08
Nitrogen%	0.60	1.04	0.56	0.93	1.31	1.17
Phosphorous%	0.41	0.46	0.36	0.39	0.53	0.61
Potassium%	0.68	0.81	0.70	0.64	0.71	0.78
Sulphur %	6.38	7.05	6.51	5.67	7.10	8.39
Protein%	3.79	6.29	3.56	5.91	7.96	7.39

Table-6: Percentage contribution of each character towards total genetic divergence in 25 genotypes

Characters	% contribution towards total genetic divergence
Ascorbic Acid(mg/100g FW)	0.67
Total Phenol Content (mg CE/100 g FW)	2.00
Total Soluble Solid(%)	0.33
Antioxidant activity(µmTE/g FW)	13.00
Flavonoid content (mg CE/100 g BW)	19.67
RSA (%)	7.67
MCA(%)	8.67
Total carotenoid(mg/100g)	3.33
Nitrogen%	9.00
Phosphorous%	8.67
Potassium%	9.33
Sulphur %	3.33
Protein%	10.00

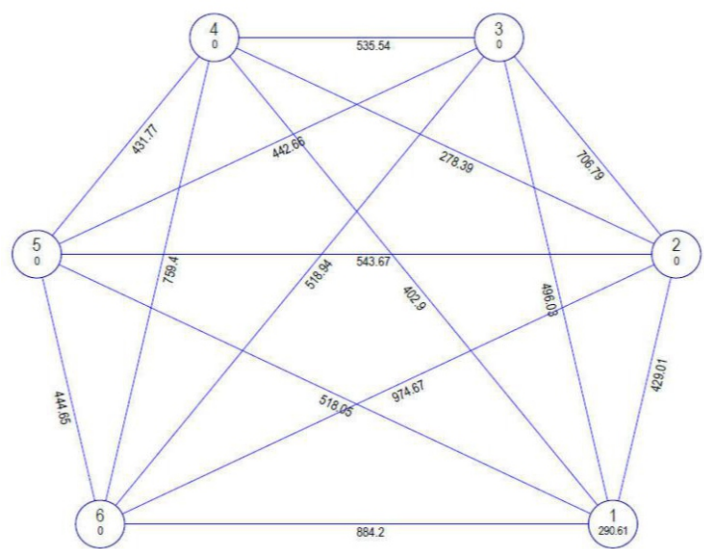


Fig. 1 : Mahalanobis Euclidean Distance (Not to the Scale)

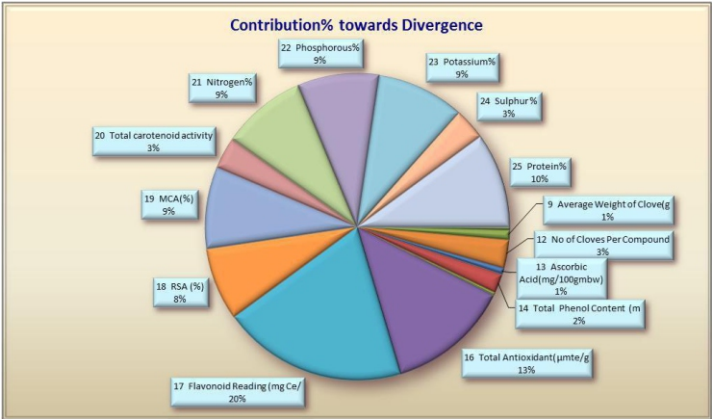


Fig. 2 : Percentage contribution of each characters towards total genetic divergence pie-chart in 25 genotypes

STATEMENTS OF DECLARATIONS

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the writing process or in the text editing.

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