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VARIABILITY IN LEAF MORPHOLOGICAL AND SIZE CHARACTERS AMONG OF *V. NEGUNDO* L. ACCESSIONS

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ABSTRACT

Vitex negundo Linn. is a woody, medicinal and aromatic shrub to a small tree having a potential medicinal property. Fifty *V. negundo* accessions from South Gujarat locations were investigated for leaf morphological variability. The design adopted was descriptive statistics for the individual parameter of fifty accessions. All fifty *V. negundo* L. accessions exhibited palmately compound leaves with a lanceolate shape. The leaf apex was acuminate, while the base was acute. The adaxial (upper) surface was consistently green, whereas the abaxial (lower) surface was uniformly light green and smooth. The leaf texture was coriaceous, and each leaf contained 3–5 leaflets across all accessions. Significant variation was observed in leaf size characteristics, including overall leaf length, leaflet length and width (for both larger and smaller leaflets), and petiole length. Principal component analysis of the variability was observed among the fifty *Vitex negundo* L. accessions which contributed to 99.98 % of the total variation and Hierarchical Clustering analysis by Ward method grouped in to four clusters. These results on leaf variability the morphological and size characteristics will contribute to the tree improvement program in South Gujarat.

Keywords: *Vitex negundo* L., medicinal plant, accessions, leaf character.

Introduction

Vitex is the largest genus in the subfamily *Viticoideae* of the family *Verbenaceae*, which comprises 250 deciduous shrub species distributed all over the world. Recently, it has been placed with *Lamiaceae* on the basis of DNA sequence data (Thomas *et al.*, 2012). According to the APG IV System of Flowering Plants Classification 2016, it has been classified under *Lamiaceae*. The genus is widely distributed in the tropical and subtropical regions of Australia, Asia, Africa with a few South American

species (Munir, 1987). *Vitex negundo* L., native to India and the is an underexploited plant adaptable to various soil textures and climatic conditions. It is found growing in almost all the parts up to an elevation of 1500 m and naturally grows abundantly in wastelands, along roadsides, near water canals and river basins, and is often used as a hedge plant around farms and homes.

This is erect, slender small tree reaches heights of 2-5 meters with quadrangular branchlets spread throughout India. Leaves are palmately compound, Petiole 2.5-3.8 cm long, 3-5 foliate, the middle leaflet

is petiolate is petiolate; in trifoliate leaf, leaf lanceolate or narrowly lanceolate, acute, entire or rarely crenate, middle leaf is 5-10 cm long and 1.6 - 3.2 cm broad, with 1-1.3 cm petiolule, remaining two subsessile; in pentafoolate leaf inner three leaflets have petiolule and remaining two subsessile; Odour is agreeably aromatic glabrous above and tomentose beneath; texture, leathery (Ladda and Magdum, 2012). The plant produces numerous bluish-purple flowers and its fruit is succulent, black when ripe, rounded and approximately 4.0 mm in diameter (Abidin *et al.*, 2015; Md *et al.*, 2022). As per study reports that *V. negundo* grows in moisture-rich open habitats and waste lands as isolated individuals. The leaves and flowers do not show any variation to classify them into different forms. The plants shed foliage and produce new foliage almost simultaneously during December–April and flower during June–November. However, certain individuals extend flowering into December and in effect delay leaf fall and leaf flushing by about a month.

Morphology currently provides most of the character used in constructing taxonomic system (Samuel and Arlene, 1986). Heywood (1967) highlighted that morphological feature have been studied so extensively by botanists in the various classes of plants that is might be assumed there is little left to learn. Studying the morphological variability of plants is crucial for any crop improvement program. Leaf morphological characteristics are essential for the identification and classification of species and specific morphotypes (Salvana *et al.*, 2019). Despite the extensive medicinal applications of *V. negundo*, there is a lack of comprehensive studies examining the combined morphological variability among its accessions, particularly in South Gujarat. Valuable insights into genetic diversity will help in selecting superior accessions of *V. negundo* thereafter for medicinal and industrial applications, and contribute to the conservation and sustainable utilization of this important medicinal plant. Hence, the current research entitled Variability in leaf morphological characters among of *V. negundo* L. accessions

Material and Methods

Study area

The South Gujarat Region of India is located between latitudes 21°14' and 22°49' N and longitudes 72°22' and 74°15' E and comprises seven districts, namely, Surat, Navsari, Narmada, Dangs, Tapi, Valsad, and Dangs covering a total geographical area of 31,495 km² Fig. 1. To the north and northeast, it is bordered by Anand and Dahod, while the state of Madhya Pradesh

lies to the east and Maharashtra, Dadra and Nagar Haveli and Daman are to the south and southeast. The Arabian Sea and the Gulf of Khambhat are located northwest. South Gujarat covers an area 17,500 sq.km the region has sub humid climate with temperature variations ranging from 6 to 45 °C, annual rainfall varying from 250 mm in the North West and to more than 1500 mm in South Gujarat.

Selection of accessions

For this study, accessions of *Vitex negundo* L. were selected from the naturally growing regions of the South Gujarat Region, which had been previously surveyed for this purpose. A detailed survey was conducted between June and July 2023 to locate and identify the trees. The collection was performed via a selective sampling strategy; each collection was allotted an accession number. During this survey, fifty accessions of *V. negundo* exhibiting favourable phenotypes were identified and collected leaf samples and marked the tree via a global positioning system (GPS) device (Make & Model: e-Trax Vista, Garmin). The geographic information of the selected accessions of *V. negundo* is presented in Fig. 1.

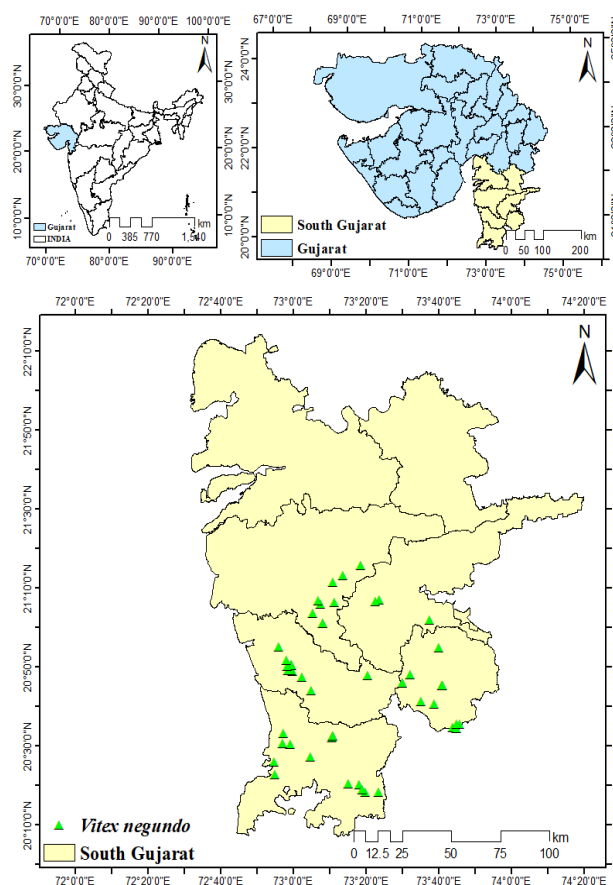


Fig. 1: Location map of selected *V. negundo* L. accessions

Leaf morphological characteristics

All collected leaf samples were carefully examined and characterized. The number of leaflets in each sample were counted. Basic morphological characteristics, such as leaf type, shape, apex, base and leaf texture were described following established leaf description (Bendre and Pandey, 1996). The colour of the adaxial (upper) and abaxial (lower) leaf surfaces was also noted. Measurements including leaf length, leaflet length and width as well as petiole length were taken using a precise scale. By counting the leaflets and detailing the morphological traits a comprehensive profile of each leaf sample was created. The use of reference book ensured consistency and accuracy in describing leaf types, shapes, apices and bases. Documenting the colours of both leaf surfaces provided additional data for distinguishing between different samples. Accurate measurements of leaflet dimensions and petiole length further enhanced the characterization, allowing for a detailed comparison of the leaf samples. This thorough examination process contributed to a better understanding of the variability and distinct features present in the collected leaves of different accessions Fig. 2.

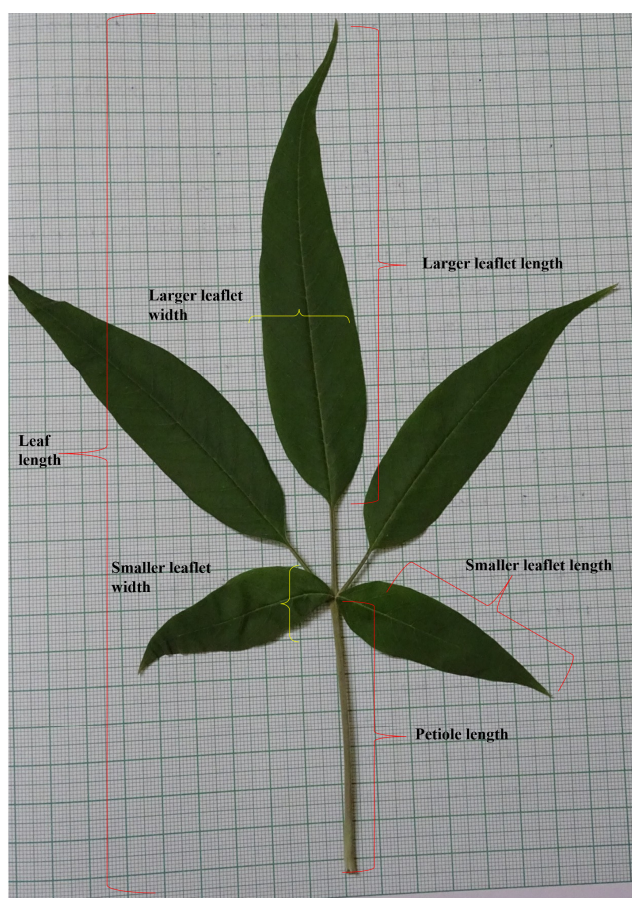


Fig. 2: Leaf morphology of *V. negundo* L

Statistical analysis

The data collected for all parameters of leaf in the study underwent descriptive statistical analysis. The results of these analyses are presented in tables which are included in the results.

Result and Discussion

Leaf morphological characters

The observations on leaf characters in different fifty accessions of *V. negundo* L. have been presented in (Table 1). Leaf characters including leaf type, shape, apex, base, colour adaxial, colour abaxial, leaf texture, entire margin and number of leaflet uniformity among *V. negundo* L. accessions was observed (Photo 4.10). The leaf type of all accessions exhibits palmately compound, the most important characters in *Vitex* species were its simple and compound palmate leaves (Bramely, 2019) and in shape all accessions have lanceolate-shaped leaves. The leaf apex is consistently acuminate across all the accessions. The leaf base is acute in all accessions. The adaxial surface of the leaves is consistently green across all the accessions. The abaxial surface was also consistently light green smooth in all accessions. The leaf texture is consistently coriaceous across all the accessions and in number of leaflet most of accession having (3 to 5) leaflet and some accession it is (2 to 5) leaflet consistently in VN 02, VN 08, VN 29, VN 30, VN 47 accessions. The data indicates a striking uniformity in leaf morphological traits across all fifty accessions of *V. negundo* L. This consistency suggests a high degree of genetic similarity or stability in these morphological characteristics among the accessions within this geographic region. Potential reasons for low variability in leaf traits could be due to several factors, including ecological similarity. The accessions are all from the South Gujarat, which may have relatively uniform environmental conditions that support the stability of these traits.

Leaf size characters (cm)

The result on leaf size characters (cm) in various accessions of *V. negundo* L. have been presented in Table 2. was observed variability collected from South Gujarat. The analysis of leaf size characteristics in fifty accessions of *V. negundo* L. from South Gujarat revealed variations in leaf length, leaflet size, and petiole length. The maximum observed leaf length was 24.6 cm in VN 14, VN 15, VN 28, and VN 31, while the minimum was 9.1 cm in VN 06. The longest leaflet length reached 17.4 cm in VN 22, whereas the smallest was 3.4 cm in VN 05, VN 20, and VN 32. Leaflet width varied from 0.9 cm (VN 05) to a maximum of 3.1 cm (VN 23, VN 25, VN 34). The longest petiole

length was recorded at 10.0 cm in VN 01, VN 15, and VN 24, whereas the shortest was 4.0 cm in VN 26. The top ten accessions based on average leaf length were VN 14, VN 15, VN 19, VN 22, VN 24, VN 26, VN 28, VN 31, VN 36, and VN 39, all exhibiting larger leaflet dimensions and robust petiole lengths.

The observed variability in leaf size characteristics among fifty accessions of *V. negundo* L. collected from the South Gujarat demonstrates the adaptability of plant. Du *et al.* (2013) noted that variation in leaflet length, width, and petiole length reflects significant morphological diversity and it is likely shaped by environmental factors such as light availability, soil composition, water accessibility, and microclimatic conditions. Our findings align with a related study by Salvana *et al.* (2019), which highlighted the importance of leaf traits in species identification within the *Vitex* genus.

The morphological variations identified in the examined leaf samples include differences in leaflet size, petiole length, leaf type, shape, apex, base, and margin. For instance, morphotype GB 58245 was classified as a medium-sized shrub growing up to 5 meters in height, with opposite, palmately compound leaves composed of three to five leaflets. The larger leaflets measured 7.3–10.0 cm in length and 1.3–1.9 cm in width, while the smaller ones measured 4.9–6.0 cm × 1.0–1.4 cm. The leaves were lanceolate with an acuminate apex, an acute base, and an entire margin. The adaxial surface was green, while the abaxial surface was light green with a smooth texture. Petiole lengths ranged from 3.0 to 3.9 cm. These detailed leaf characteristics have been extensively documented in botanical literature to provide a comprehensive understanding of taxonomic classifications (Ellis *et al.*, 2009).

Leaf traits are generally regarded as genetically determined features (Roth *et al.*, 2001), although their

expression can be influenced by environmental interactions. Leaf morphometric analyses are widely applied to characterize and elucidate infraspecific variants and morphotypes. Quantitative assessments of phenotypic variation within and between species have proven effective in plant morphometric studies (Pearson, 1901). Md *et al.* (2022) proposed that leaf architecture could serve as a reliable taxonomic marker for distinguishing species, subspecies, and varieties within the *Vitex* genus, potentially prompting revisions in the classification of certain taxa.

Furthermore, Okocha *et al.* (2024) documented considerable intraspecific morphological diversity among *V. doniana* accessions, reinforcing the relevance of leaf characteristics in plant systematics. Similar variability in leaf size has been reported across multiple *Vitex* species, including *V. gamosepala*, *V. glabrata*, *V. longisepala*, *V. millsii*, *V. negundo*, *V. pinnata*, *V. quinata*, *V. trifolia*, and *V. vestita* (Md *et al.*, 2022). Salvana *et al.* (2019) also observed significant leaf size variations in *V. negundo* L., further emphasizing the importance of leaf traits in species differentiation. Other research has corroborated these findings in various plant species. For example, Hashemi and Khadivi (2020) documented morphological variability in *Morus alba*, while Anushma and Sane (2018) reported similar variations in *Syzygium cumini*. Likewise, Imorou *et al.* (2022) observed comparable diversity in *Erythrina sepium*, and Jamwal *et al.* (2022) identified significant leaf size variations in *Justicia adhatoda*. Significant variation was observed in the leaf size characteristics among *V. negundo* L. accessions from the South Gujarat. This included differences in overall leaf length, the length and width of the larger leaflets, the length and width of the smaller leaflets and the petiole length. These studies highlight the widespread occurrence of leaf size variability across different plant taxa, emphasizing its significance in morphological and taxonomic research.

Table 3 : Principal component analysis of the leaf size characters variability

Parameter	PC1	PC2	PC3	PC4	PC5
LLL	0.43096	0.68925	-0.39986	0.41253	0.09562
LLW	0.81043	0.06732	-0.02887	-0.43889	0.38107
SLL	0.77395	0.16167	0.33356	-0.06412	-0.50940
SLW	0.55281	-0.62498	0.27133	0.44374	0.18240
PL	-0.26626	0.49282	0.79784	0.06677	0.21265
Eigenvalue	1.818	1.139	0.982	0.568	0.492
Percent	36.36	22.78	19.64	11.36	9.84
Cum percent	36.36	59.14	78.78	90.15	100.00

Principal component analysis of the variability was observed among the fifty *Vitex negundo* L. accessions collected from the South Gujarat across all morphological leaf parameters, revealed five principal components, four of which were reported to have eigenvalues of 1.818, 1.139, 0.982, 0.568 and 0.492 respectively Figure 3., which contributed to 99.98% of the total variation Table 3. The loading matrix is given in Table 3. and resulted in a significant positive association of the first principal component with leaf length, the length and width of the larger leaflets, the length and width of the smaller leaflets and except the petiole length. Principal component analysis (PCA) is a statistical technique that reduces large datasets with numerous correlated variables into a smaller set of new

variables while retaining most of the variation present in the original data. This simplification makes the interpretation of results more practical and meaningful. PCA focuses on retaining only a few key components that account for the majority of the variation while discarding less informative components.

The scatter plot revealed minor variations in grouping compared to the cluster analysis, as illustrated in Figure 4. The PC1 explain the 36.40% of the variance, while PC2 explains 22.80% of the variance. Most of the accessions are clustered around the center but some like VN 05, VN 50, VN 26, VN 37 and VN 02 were positioned far away, indicating variability among the accessions.

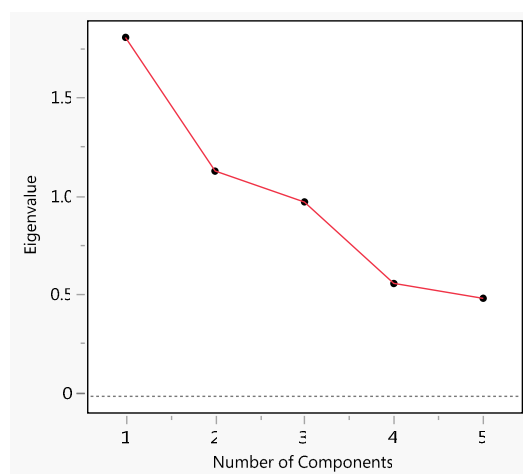


Fig. 3: Scree plot based on various leaf size characters among *V. negundo* L. accessions

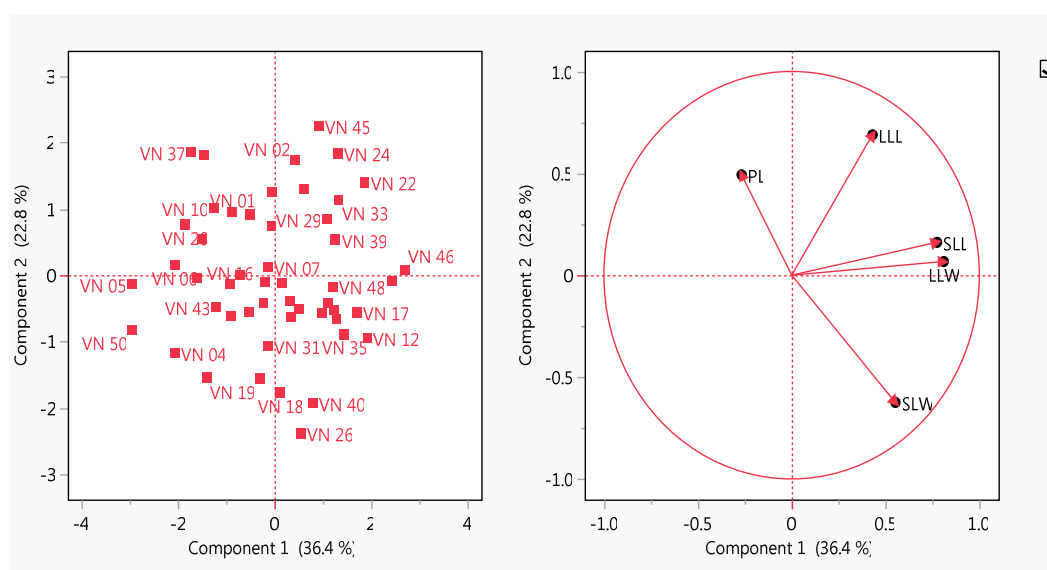


Fig. 4 : Scatter plot of fifty accessions based on two principal components for different leaf size characters among *V. negundo* L. accessions

Hierarchical Clustering analysis by Ward method

The *V. negundo* L. accessions were separated into four clusters in the cluster analysis on the basis of their leaf morphological size characters Fig. 5 and Table 4.

Table 4 : Cluster means leaf size characters among *V. negundo* L. accessions

Cluster	No. Accessions	LLL	LLW	SLL	SLW	PL
1	6	13.0767	2.35667	6.0967	1.5067	7.0667
2	5	12.9520	2.55600	6.1440	1.7520	5.9120
3	24	12.6508	2.31583	5.5542	1.9133	6.4258
4	15	12.4560	2.20400	4.9947	1.5093	6.7067

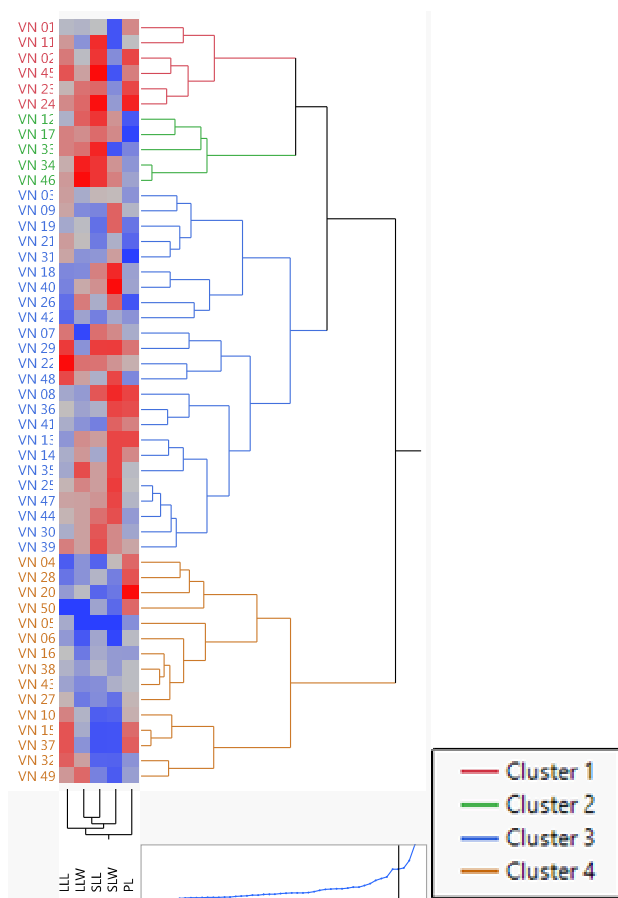


Fig. 5: Hierarchical cluster analysis (Ward method) based on leaf size characters of fifty accessions of *V. negundo* L.

Cluster I comprised six accessions, Cluster II comprised 5 accessions, Cluster III comprised 24 accessions and Cluster IV comprised 15 accessions. Hierarchical cluster analysis (HCA) is a statistical method used to simplify complex multivariate data by grouping it into smaller, more manageable subsets. It

classifies data into distinct groups, with the data within each group being considered similar.

Conclusion

The leaves of all fifty accessions of *V. negundo* L. are palmately compound, with a lanceolate shape. The leaf apex is acuminate, while the base is acute. The adaxial (upper) surface of the leaves consistently exhibits a green coloration, whereas the abaxial (lower) surface is uniformly light green and smooth. The leaf texture is coriaceous and each leaf bears 3 to 5 leaflets across all accessions. The variability was observed among the fifty *Vitex negundo* L. accessions collected from the South Gujarat across all leaf morphological characteristics indicating strong potential for selection and improvement of this species.

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Conflict of interest

The author declares no conflict of interest.

Author Contribution

ASG: Design, conduct field surveys and data, sample collection from various locations, laboratory analysis, preparation of graphs and tables, writing of the manuscript for publication. SKJ, BD, YBG, DP, SKS: Research methodology, Supervision, data analysis. All the authors have read and agreed with the published version of this manuscript.

Table 1 : Variability in morphology of leaf characters among *V. negundo* L. accessions from South Gujarat

[illegible]

Table 2 : Variability in leaf morphological characters among of *V. negundo* L. accessions from South Gujarat

Accessions Number	leaf length (cm)	Longer leaflet length (cm)	Longer leaflet width (cm)	Smaller leaflet length (cm)	Smaller leaflet width (cm)	Petiole length (cm)
	Range	Range	Range	Range	Range	Range
VN 01	10.2 - 22.8	10.6-14.4	1.9-2.6	4.6-6.3	1.3-1.5	5.5-10.0
VN 02	14.6 - 23.4	11.6-16.1	2.1-2.5	5.3-7.2	1.3-2.0	6.0-8.0
VN 03	12.4 -23.1	11.2-15.7	2.0-2.6	4.0-7.0	1.4-2.2	5.0-7.2
VN 04	14.5 -20.8	9.7-12.3	1.7-2.5	3.8-.5.7	1.5-2.1	6.5-8.0
VN 05	10.2 - 23.4	10.5-14.5	1.7-2.4	3.4-5.4	0.9-2.0	4.5-8.0
VN 06	9.1 - 22.2	10.6-14.0	1.8-2.4	5.0-6.0	1.2-1.6	5.4-8.0
VN 07	12.4 -23.4	11.4-15.3	1.5-2.7	5.0-7.2	1.5-2.2	6.0-7.0
VN 08	14.5-20.8	10.7-14.6	1.7-2.6	5.2-7.4	2.0-2.2	6.0-8.0
VN 09	9.6 -23.4	11.6-14.2	1.8-2.5	4.0-6.0	1.5-2.1	5.2-7.5
VN 10	11.6 - 22.6	12.1-15.1	2.0-2.5	3.4-5.8	1.2-2.0	6.4-7.0
VN 11	9.8 - 22.5	11.6-14.8	1.8-2.4	5.4-7.4	1.3-1.5	5.6-8.0
VN 12	12.4 - 22.4	11.0-14.1	1.9-3.0	5.3-7.2	1.5-2.0	5.1-6.0
VN 13	11.6 - 20.8	10.6-13.2	2.0-2.7	4.8-6.2	1.4-2.5	5.4-9.0
VN 14	12.6 - 24.6	11.6-13.1	1.9-3.0	4.0-6.4	1.5-2.4	6.0-7.2
VN 15	10.2 - 24.6	12.0-15.0	2.0-2.6	4.0-5.0	1.0-2.0	5.5-10.0
VN 16	12.4-22.4	10.1-16.0	1.8-2.5	5.0-6.2	1.2-2.0	5.0-8.0
VN 17	14.5-20.8	12.0-14.6	2.2-3.0	4.5-7.2	1.5-2.2	4.9-6.0
VN 18	10.8-23.4	10.0-12.5	2.0-2.4	5.2-6.4	2.0-2.2	5.4-7.5
VN 19	14.3-24.3	11.6-13.0	1.9-2.6	4.0-6.0	1.5-2.1	5.4-6.0
VN 20	14.5-22.4	10.2-15.1	2.1-2.5	3.4-6.0	1.2-2.0	7.0-8.0
VN 21	13.4-23.1	11.8-14.2	2.0-2.6	3.4-6.0	1.4-2.0	4.6-7.6
VN 22	14.3-23.5	12.0-17.4	2.4-2.6	4.0-8.0	1.4-2.2	5.0-9.0
VN 23	14.6-22.1	11.0-15.2	2.1-3.1	5.0-7.0	1.4-2.2	6.0-9.0
VN 24	12.4-23.4	12.0-15.0	2.1-3.0	5.3-7.5	1.3-2.0	5.5-10.0
VN 25	14.5-20.8	11.2-16.0	2.1-3.1	5.0-6.2	1.6-2.2	6.0-7.2
VN 26	10.6-23.4	10.0-13.0	2.0-3.0	4.0-6.4	1.5-2.1	4.0-7.2
VN 27	11.6-23.1	10.5-15.0	1.7-2.5	4.0-6.0	1.0-2.0	6.0-7.0
VN 28	11.5-24.6	10.0-14.0	2.0-2.5	5.0-6.0	1.2-2.0	6.0-8.0
VN 29	12.4-22.4	12.8.0-15.3	1.8-2.7	5.0-7.2	1.8-2.2	5.5-8.0
VN 30	12.4-20.8	11.0-14.6	2.0-2.6	5.2-7.4	1.4-2.2	6.0-6.8
VN 31	12.6-24.6	11.6-14.2	1.9-2.5	4.0-6.4	1.4-2.1	5.0-6.0
VN 32	12.4-21.9	12.6-15.1	2.1-2.5	3.4-5.8	1.2-2.0	5.6-6.5
VN 33	12.4-22.4	12.0-15.0	2.1-3.0	5.4-7.4	1.3-1.5	5.5-7.0
VN 34	14.5-20.8	12.0-14.1	2.4-3.1	5.3-7.2	1.5-2.0	5.1-6.7
VN 35	10.8-23.4	11.0-13.2	2.5-2.8	4.8-6.2	1.4-2.5	5.2-8.0
VN 36	14.6-24.3	12.0-14.0	2.0-2.6	4.0-6.7	1.5-2.4	6.0-8.1
VN 37	14.5-21.6	12.0-15.0	1.7-2.6	4.0-5.0	1.0-2.0	6.5-7.6
VN 38	13.4-23.1	10.1-15.0	2.0-2.5	5.0-6.5	1.2-2.0	4.5-9.0
VN 39	12.6-23.5	12.0-14.6	2.2-2.8	5.0-7.2	1.5-2.2	6.0-7.0
VN 40	14.6-22.4	10.0-12.5	2.1-2.5	5.2-6.1	2.0-2.2	5.9-6.8
VN 41	11.3-20.4	11.8-13.0	2.0-2.5	4.0-6.6	1.5-2.1	6.0-8.0
VN 42	10.5-23.4	10.0-12.1	2.0-2.5	3.4-7.0	1.2-2.0	4.7-7.0
VN 43	14.3-24.3	11.8-12.6	1.8-2.6	4.0-6.0	1.4-2.0	5.0-8.0
VN 44	14.5-18.9	12.0-13.5	2.0-2.5	5.0-7.1	1.4-2.2	6.0-6.5
VN 45	13.4-23.1	12.8-15.0	1.9-2.8	5.4-8.0	1.3-1.5	5.8-8.0
VN 46	12.6-23.5	11.1-16.0	2.4-3.1	5.3-7.2	1.5-2.0	6.0-7.0
VN 47	15.6-24.3	11.6-14.2	1.8-3.0	4.8-6.2	1.4-2.5	5.0-7.2
VN 48	14.4-22.1	12.6-15.1	2.1-2.5	4.0-6.7	1.5-2.4	5.0-7.0
VN 49	13.4-23.1	12.0-14.3	2.1-3.1	4.2-6.8	1.1-2.2	5.0-7.1
VN 50	11.4-22.7	10.0-12.1	1.8-2.2	5.0-6.0	1.2-2.2	6.0-8.1
Mean	17.61	12.67	2.31	5.51	1.72	6.53

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