Zone Based Phytochemical Variability In Different Genotypes Of Moringa Oleifera Lam. Using Ftir Spectroscopy

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Abstract: Infrared (IR) spectra of particular plant species are highly specific fingerprint-like signature which are used to differentiate, classify, and identify diverse plant species. Plant specific IR spectra are useful to detect functional group or structure. The spectral traits can be systematically extracted from the typically broad and complex spectral contours applying resolution enhancement techniques, difference spectroscopy, and pattern recognition methods such as factor-analysis and cluster-analysis, and artificial neural networks. The spectral pattern varies with effect of climatic conditions such as stress, salinity, drought and other factors that's affecting the plant development. FTIR techniques proved to be an effective strategy for identifying the species and for determining their geographical distribution, especially in the assessment of *Moringa* quality for use in raw herbal medicines. In this study, results of FTIR analysis confirmed the presence of phenol, alkanes, aldehyde, secondary alcohol, amino acid, aromatic amines and halogen compound between 500-4000 cm-1 region and provide a platform for providing variability in spectra of 57 *M. oleifera* aceessions that collected from different geographic locales of India. Based on the spectrum value the factor analysis and cluster analysis were also done.

Index Terms: Phytochemical, *Moringa oleifera,* Petroleum ether, Fourier transforms infra-red spectroscopy

1 Introduction

Moringaceae are old-world perennial soft-wood trees that are distributed in tropical regions of the world. These trees indigenous to the western and sub Himalayan tracts, including India, Pakistan, Asia minor, Africa, and Arabia [1], but have now spread to other regions of the world, including the Philippines, Cambodia, Central America, North and South America, and the Caribbean Islands [2].This family serves as sources of natural antioxidants including ascorbic acid, flavonoids, phenolics, and carotenoids [3,4]. *Moringa oliefera* Lam. only be allied to the family Moringaceae is the most extensively used medicinal plant, popularly known as Drumstick tree. *Moringa* has 2c genome size of 1.2 pg and this plant is a true diploid with 2n = 28 [5]. Being one of the most nutritive plant, phytochemical analysis and antioxidant activity has been reported in various studies [6, 7, 8, 9]. The knowledge of *Moringa* diversity even within our country is very meager. Considering its highly nutritive and medicinal value it is very crucial to study chemoprofiling of *M. oleifera* within the country. Plants are the excellent sources for some pharmaceutical components and secondary metabolites which are used to cure several ailments of the individuals with no side effects and confer special characteristics and properties to the herbs [10]. Phytochemicals especially polyphenols constitute a major group of compounds that act as primary natural antioxidants [11, 12].

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More than four thousand polyphenols are found in vascular plants. Thus, identification of the nature of phytochemical constituents will ensure about knowledge of different functional groups responsible for their therapeutic properties. Several techniques such as Chromatography and spectroscopic are used to identify the presence of phytoconstituents in medicinal plants. Out of which chemoprofiling that establishes a characteristic chemical pattern for a plant material and used to characterize the closely related plant species [13]. There are several metabolic profiling and fingerprinting techniques have been established. It includes 'Fourier transforms infra-red spectroscopy' (FT-IR), 'High Performance Liquid Chromatography' (HPLC), 'nuclear magnetic resonance spectroscopy' (NMR), 'Liquid Chromatography Mass-Spectroscopy' (LC-MS) and Gas Chromatography Mass-Spectroscopy' (GC-MS). However, simple and cost beneficial tests for detecting phytocomponents in selected plant are Spectroscopic methods like UV-Vis and Fourier Transform Infrared (FTIR) together or alone can be used to specify the phytoconstituents in plant inividuals. FTIR analysis is most effective and validates technique than spectrophotometry (UV-Vis), to recognize functional groups of biologically active components [14,15]. This work incorporates the phytochemical fingerprinting of *M. oleifera* using FTIR. However there are no many reports on the analysis of this kind of plant by using FTIR. This non-destructive method has been used in a realist form to analyze leaves, seeds, flowers and roots in several plants. In our work, we have determined the infrared absorption from leaves of *Moringa* as another kind of technique to investigate the main functional groups present.

2 MATERIAL AND METHODS

2.1. Plant materials

Leaf samples of various accessions of *M. oleifera* were collected from six different agro-ecological regions of Rajasthan, Uttarakhand, Uttar Pradesh, Madhya Pradesh and Goa as shown in **Fig.1**. Total 57 samples of *Moringa* for phytocahemical variability and one sample of *Acacia*

nilotica taken as outgroup from Banasthali. Latitude and longitude of every plant is also recorded during the collection of plant sample of different regions. The chemicals used for FTIR were of molecular biology grade from Banglore Genei Pvt. Ltd, India.

2.2. Extraction

FTIR analysis of different leaf extracts of *M. oleifera* was done by using method of Elangovan et al ., 2015 [16]. The dried and powdered leaves of all samples were soxhlet extracted with petroleum ether solvents for 8 hours. The extracts were concentrated and further used for FTIR analysis. The FTIR spectra of functional groups detected on a FTIR and the characteristic peaks of functional group were recorded individually and compared all the spectra of different regions.

3. RESULTS AND DISCUSSION

3.1 FTIR analysis

The major peaks identifiable in *M. oleifera* showed in *Fig.2*: 1. A broad band around 3619 and 3000 cm-1 which are associated with phenols and alcohols with hydrogen bond - OH stretch, which usually appears between 3500-3200 cm-1. A peak between 3500- 3100 cm-1 which could also be due to N-H stretch from a primary or secondary amine or amide in which R could be alkyl or aryl. 3. A similar band around 3000- 2800 cm-1 due to C-H stretch from a hydrocarbon. 4. In some samples but not in all peaks observe at 2361cm-1 that represents P-H phosphine band. Between 1750 and 1625 cm-1, aliphatic C=O bonds due to aliphatic ketones or esters are identifiable. 6. In the finger print region, there are peaks around 1500-1375cm-1. There appears to be –OH band at 1462.04 cm-1 and N–O symmetric stretch from aliphatic nitrogen containing organic compound. There is also a C=C-H from 900-675.09 cm-1 pointing to C-C stretching and C=C bonds in aromatic rings 7.The absorption at 1256. 40 cm-1 due to a C-H wag (CH2X) or stretch from an alcohol or phenol. This has been evident in the spectrum of *M. oleifera* leaf. When intermolecular interactions are weak, the numbers of molecules are small, and narrow infrared bonds are observed. Most of the frequencies are group frequencies which tell the presence or absence of specific functional groups in a sample. The peaks therefore, between 3500-3200 cm-1, 3500- 3100 cm-1, 2750-250 cm-1 and at 900 – 675 cm-1 are diagnostic marker for the presence of OH, NH, C=O and C=C functions respectively. The C-H stretch associated with H-C=O usually differed a little in frequency because of the inductive effect of oxygen attached to the carbon atom also attached to the hydrogen atom, thereby making it weaker. Thus, in the spectrum of the leaf of *M oleifera* a C-O stretch at 1256.40 cm-1 may be used to confirm the presence of – OH group in phenol or alcohol which usually appears between 3500-3200 cm-1. The broad band, therefore, in this region of the spectrum is diagnostic of the presence an OH function. The results of IR analysis also reveal that, the components of *M. oleifera* leaf could be aliphatic or aromatic. It may therefore be inferred that aromatic or aliphatic alcohols or phenols, amine, ketones, esters and some nitrogen containing compounds are some of the constituents of the leaf of this plant. However, because of the fingerprint region which has a pattern that is specific for every molecule, the presence of OH function and N-O stretch, suggest that aromatic or aliphatic phenols or alcohols and nitrogen containing molecules are major components of the *M. oleifera* leaf that was studied. However, because the spectrum is from a mixture, the fingerprint region cannot be particularly assigned to any specific molecule. In dendrogram two clusters form, sample MO- 36 present in different cluster, act as outlier and all samples other than MO- 36 were grouped into the different cluster because all samples of same plant have same functional groups. The PCA data and dendrogram of FTIR bands contained in the 500-4000 cm-1 region obtained during FTIR analysis as illustrated by Fig.3 and Fig.4 respectively.

3.2 Discussion

In our study, *Moringa* samples of various geographic regions have approximately the same functional group but their band intensities and positions exhibited several variations Fig.2. In addition, many weak FTIR bands were noticed which may be employed as marker for the samples originating from several agroecological regions [17]. It reveals that genotype and environment interactions also induce the diversification of chemical components, other than genotypic differences [18, 19, 20, 21]. Nowadays, FTIR has become a method of versatility in plant bioscience of molecular biology [22], ecology [23], physiology [24] and agriculture [25]. This technique has also been utilized in plant classification and identification at levels of genus, section, species and even cultivar [26, 27, 28, 29]. These investigations have shown that FTIR provide a substantial amount of information for determining *M. oleifera* of various geographical origins. FTIR spectroscopy has been shown to be a useful tool for discriminating and categorizing closely related species. Li et al., (2010) reported in his studies that FTIR could effectively utilize for identifying phylogenetic relationships between flowering plants [30]. Wang, 2012 used this technique to examine the species in *Hypericum* and *Triadenum* [31]. In *Moringa* also exploited this technique effectively. Gorgulu in his research indicated that FTIR spectroscopy could be efficiently applied to discriminate genera Ranunculus, Astragalus and Acantholimon [32]. In any sample where hydrogen bonding occurs, the number and strength of intermolecular interaction varies greatly within the sample, causing bonds in the sample to be particularly broad [33]. The characterization of petroleum ether extract of *M. oleifera* leaf reveals the presence of C=O, C-O, C=C etc band stretching, suggesting that components of *M. oleifera* leaf may be aromatic or aliphatic. In the IR spectrum, the bands at 3000-2800 cm-1 which are due to C-H stretch from a hydrocarbon correspond to the findings of Bajia, 2007 on the same study [34].

4. Conclusion

The result of analysis using FTIR spectroscopy showed that various functional groups were present in extract of *Moringa* plant. All samples have approximately same functional groups but variation in several main bands can't be ignored because their band intensities and positions exhibited several variations. In future, medical practitioner can be

using these variations for identification of good yielding genotypes from several areas for medicinal purpose.

5. CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

6. Acknowledgment

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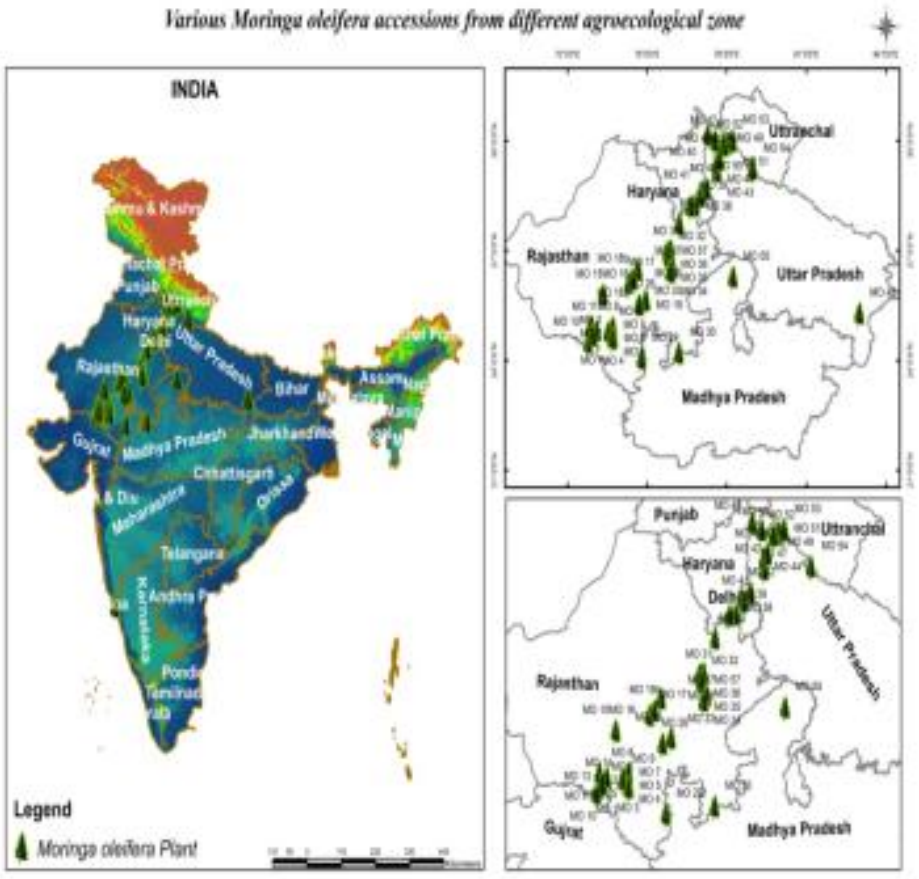

Fig.1: *Location representing regions of sample collection from different agro-ecological zone.*

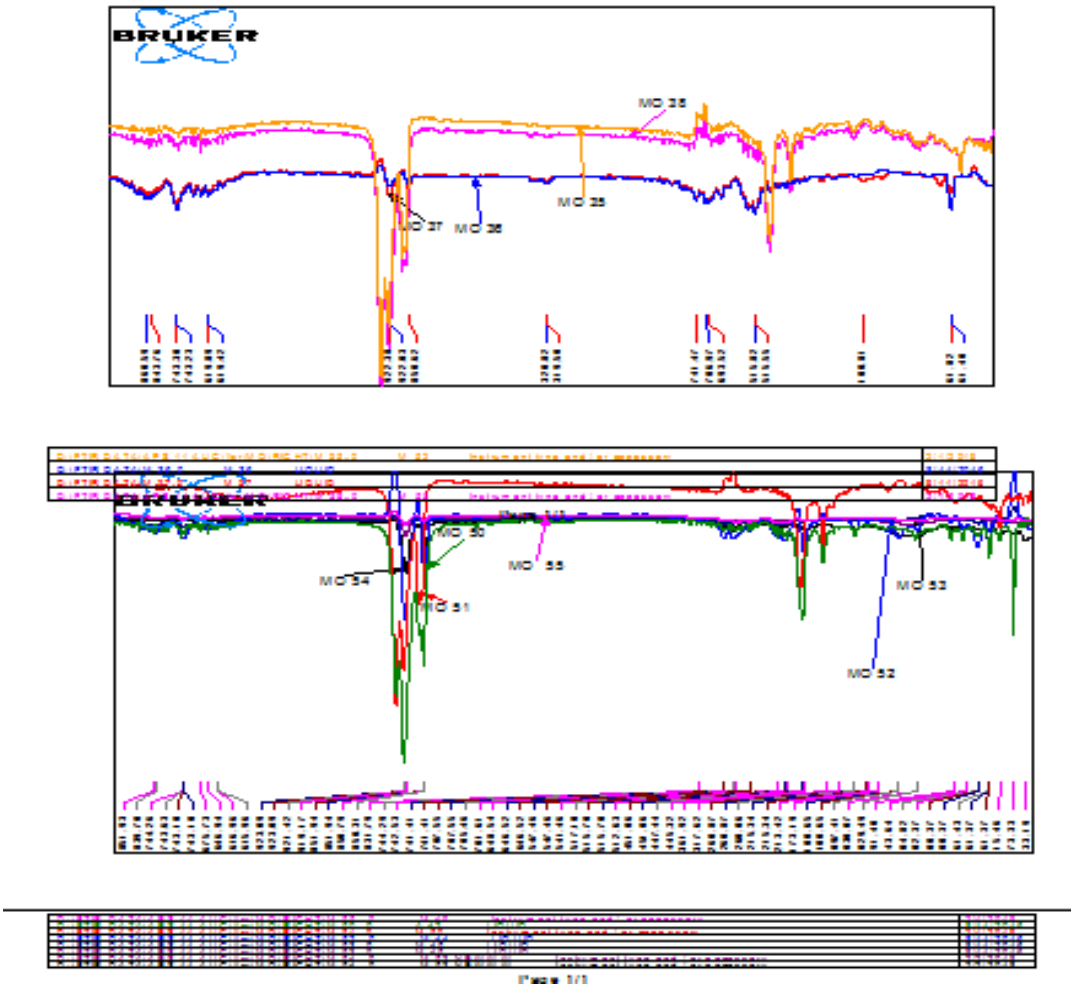

Fig.2:
Spectrum of different *Moringa* samples from different geographic locales of India

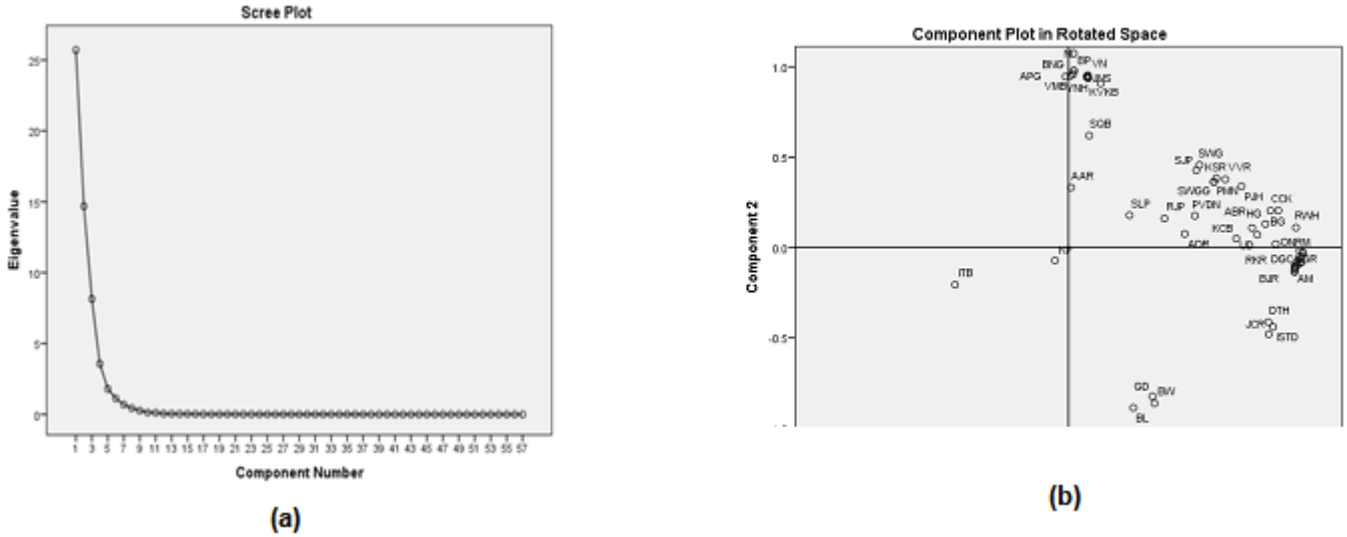

Fig.3: *PCA analysis based on FTIR data. (a) Scree plot,and (b) Component Plot*

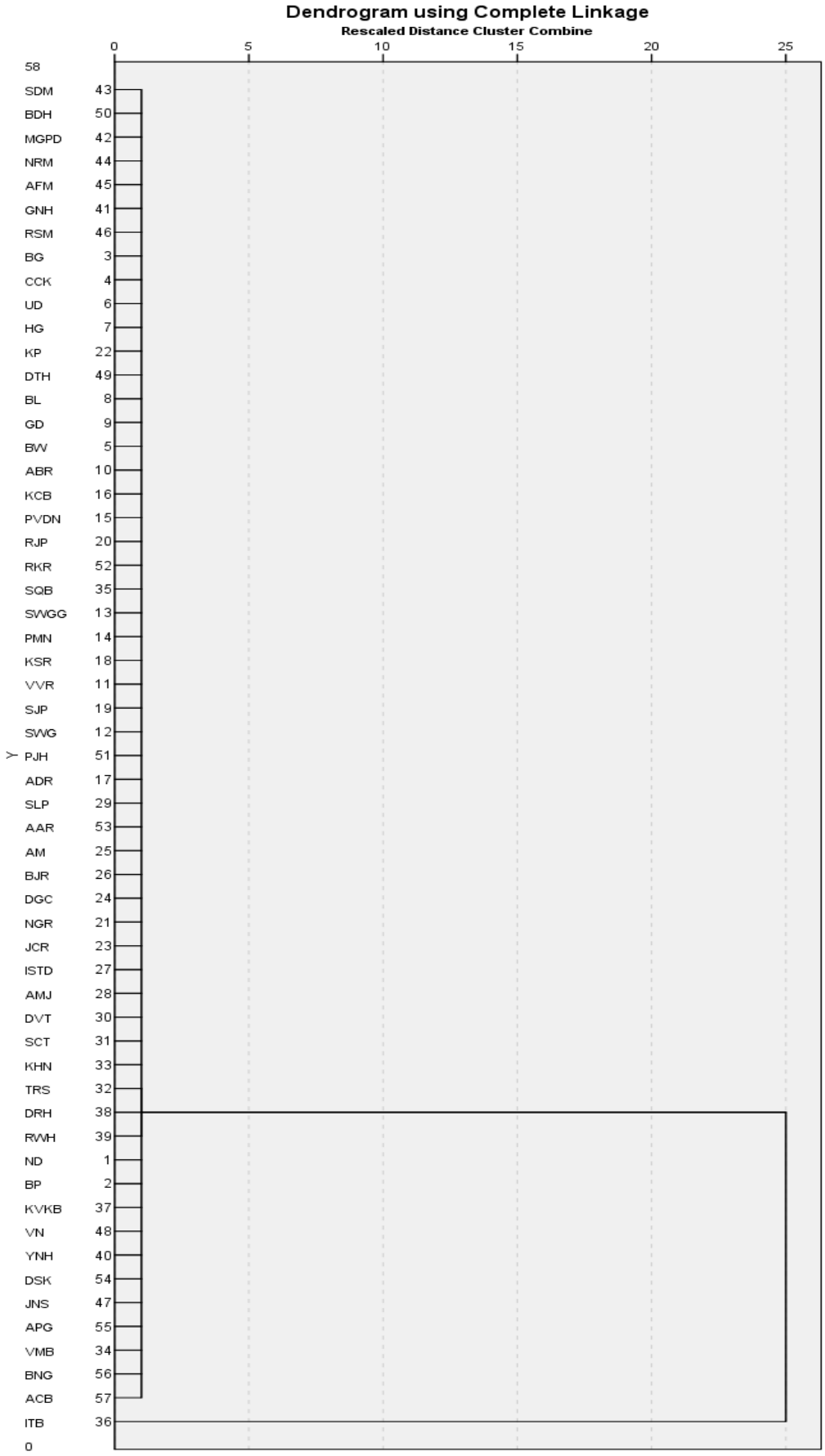

Fig.4: *Dendrogram based upon FTIR functional groups among genotypes of M. oleifera.*