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Characterization of Pungent Red Chilies (*Capsicum* spp.); Cultivars of Manipur and Nagaland, part of Indo-Burma Mega Biodiversity Hotspot

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ABSTRACT
Chili has high endemism in Manipur and Nagaland. Present study is an attempt to collect data on compositional inventory from locations known for chili cultivation in the region. Chemical variability was noted though there was no major soil nutrients deficiency. Out of the ecologically adapted collection of 33 location fruit samples, 22 were in-situ and 11 were ex-situ. The results obtained herein conclude that these cultivars have marked differences in pungency. C. chinense from Saijang had highest capsaicinoids contents compared to other locations. Saponins from fruits of each cultivar have unique chromatographic fingerprints, useful to identify even at physically modified form.
Keywords*: Compositional differences, Physically modified forms; Fingerprints, Soil nutrients.*

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INTRODUCTION

Manipur and Nagaland, the Northeast States of India traditionally cultivate chilies (*Capsicum spp.*) as subsistence-based farming, and local names vary with the place to place. Farming is depending on the traditional rain-fed system, with no fertilizer input. Fruits collection, drying and seeds preserving, is totally based on traditional methodology. Fruits used for spice, ethnomedicines and smeared on fences or burned to smoke to keep wild elephants away for personal safety [1]. Every farmer surveyed at Nagaland was cultivating the Naga Mircha (*C. chinense*) on a priority basis. In Manipur additional chilies, namely Meitei Morok and Yelhlong Morok (landrace varieties of *C. frutescens*), and Morok (*C. annuum*) have equally planted along the Umorok (*C. chinense*, known as 'Naga Mircha' in Nagaland). The hilly slopes were good for cultivation of Umorok and Yelhlong Morok, while Meitei Morok was in the plains of Manipur. Morok was cultivated altogether in both hill slopes and plains of Manipur. In Nagaland mainly *C. chinense* was preferred, only a few plants of *C. annuum* were found. Farmers were least concern for its care, utility and trade. The region is known as one of the first places where human had used fire for agriculture, still inhabited by more than 225 tribes out of the total 450 tribal communities of the country [2]. Most of these tribal groups are settled in more remote, mountainous parts of the region, economically poor but rich in cultural values retains traditional crops for their dietary and religious rituals [3]. Traditional crop varieties and practices are the identities of the tribe(s) inhibiting the in the region. These varieties possess stress tolerant properties, a factor for tribal like to retain these landraces as informed during the survey by all.

State Manipur and Nagaland, India, is a part of Indo-Burma mega biodiversity hotspot, characterized by distinct seasonal weather patterns, dry, cool winds from continental origin during Northern winter months but on spring wind direction reverses, brings abundant rain. A wide diversity of ecosystem, varieties of localized vegetation and seasonally inundated grasslands are the salient features of the region [4]. Each species has its highest fitness at its native [5], but *C. annuum* an exception as a native of South America introduced to Europe after Columbus voyage and finally in Africa and Asia [6]. Asian climate is the most favorable for this species, as it has above 85% of world production [7]. The chemical composition of plants varies with local geo-climate, seasonal changes and external conditions such as light, temperature, humidity and soil fertility, which affect the quality and properties of plants when used for pharmaceutical, nutritional and industrial applications. Plants of the same species but occurring in different geographical sites may differ significantly in the chemical composition, particular in case of secondary metabolites [8].

According to all farmers during survey *C. chinense* is a shade loving crop, so usually cultivated against the bamboo fencing. The nutritional health benefits of *C. chinense* fruits, either green or red, were described and praised by every farmer for gastrointestinal well-being without any side effect. Yelhlong Morok is used to increase the pungency of Meitei Morok or Morok but rarely used alone, was also informed. Meitei Morok was confined in Meitei community dominated areas in the valley of Manipur while Yelhlong Morok in the sloppy elevations of district Bishnupur only.

There were no geographical boundaries as barriers for limiting gene flow among the population, selected for the present study. The objectives were to; (a) Establish the pungency diversity among of *Capsicum* cultivars, (b) Determine the variation in pungency with a change of location, (c) A relation between pungency concentration and soil nutrients, if any and (d) Co-relate the traditional and molecular therapeutic knowledge. We have examined the chemical composition of *C. annuum*, *C. chinense* and *C. frutescens* (Meitei Morok and Yelhlang Morok, the landrace varieties, respectively) from the region, both *in-situ* and samples from street vendors, along soil nutrients from the *in-situ* sampling site.

MATERIAL AND METHODS

Chemicals

Solvent and reagents were purchased from RankemTM, part of Avantor (17 Floor, Building No. 5, Tower C, DLF Cyber City, Phase-III, Gurgaon-122002, Haryana, India). Reference standards were procured from ChromaDex.

Sampling

Chili distribution pattern and its extent in Manipur and Nagaland were investigated from July 2013 to mid-Nov. 2014. Only the first year crops were selected for *in-situ* sampling to ensure the consistency of age of fruits. The study was conducted in districts Dimapur, Kohima, Peren (State Nagaland), Bishanupur, and Imphal West (State Manipur), locations known for the cultivation of chilies in the region for domestic use.

Fig. 1: Sample collection sites

Details of accessions, collection sites (Fig.1), and geographic coordinates were documented. Ten healthy individual fruit-bearing plants of similar growth and age status were selected from distance of 1.5 m in Jhum fields and ¼ m in Valley (due to space problem as cultivated either on backyard or kitchen garden) from a particular site. Mature fruits *in-situ* and soil from four directions of the same plant (0-150 mm depth) were collected, mixed all as one individual sample from the site, respectively. Thus, there were total 22 *in-situ* samples (Table-1) and 11 from market (Table-2) along 22 soil samples (Table-3). Market samples for a particular location were from 11 different sources, mixed all as a representative sample of the site. The source of samples in Nagaland was mainly the 'Jhum field', where Paddy was the main crop, and under small segregated area chili was planted. These Jhums were surrounded by thick patches of natural forests. During sampling farmers and vendors were approached for information on personal details and documented on the 'Passport Data Sheet', National Bureau of Plant Genetic Resources (NBPGR), New Delhi-110012, after taking their prior informed consent.

The fruit samples were dried under vacuum at 35°C and grounded to powder and analysed, Voucher specimens samples from all sites were identified by Dr. D. K. Hore, Plant Taxonomist, Senior Consultant, IBSD-Imphal, and were deposited at the IBSD-Imphal Herbarium (IBSD/M-217-249). The soil samples were air-dried, ground and sieved using a 0.5 mm stainless sieve, and stored at 5°C under laboratory condition until analysis.

Extraction and quantification of capsaicin and capsaicinoids

Extraction and quantification were carried-out in triplicate for each sample. Chili fruits, powdered after drying, accurately weighed and extracted with three times (wt/v) acetone, HPLC grade, with stirring on a magnetic stirrer for three hours each, at thrice. Filter and mixed all the filtrate and finally make up to 100 ml. Quantification using Waters HPLC, C-18 column (250 x 4.6, 5µ, Waters XTerra®), mobile phase water: acetonitrile :: 50:50 (v/v), flow rate; 1.0 ml/min, UV detection at 281 nm, injection volume 20µl after passing through 0.45 µ filter. The standard solutions (of capsaicin and dihydrocapsaicin, respectively) were also injected at regular intervals between sample injections to confirm the retention time, which were previously used to plot the calibration curve. The sum of capsaicin, dihydrocapsaicin and other capsaicinoids was expressed as total capsaicinoids.

The linearity of method was determined using series of diluted standard solutions of the stock standard with a range from 1.0 - 30 µg/ ml, for both capsaicin and dihydrocapsaicin respectively. Linearity was found 1.0 - 26 µg/ ml in the curve plotted. Retention time and peak area were criteria for repeatability (intraday precision, deduced from 10 replicates, n=10) and reproducibility (inter-day precision, six analysis daily, n= 3 x 6) for both compounds. The relative standard deviations (RSDs) were less than 1% and 1.3%, respectively. The limit of detection (LOD) (signal/ noise = 3) and limit of quantification (LOQ) (signal/ noise = 10) of the capsaicin and dihydrocapsaicin were same, 0.5 µg/ ml and 1.0 µg/ ml, respectively. A recovery experiment was performed by adding a specific quantity of capsaicin and dihydrocapsaicin, respectively, in a quantified sample, the average percent recovery of capsaicin and dihydrocapsaicin was 98.37±2.89% and 98.71±2.13%, respectively. The stability of these two compounds in the sample solution was evaluated using relative peak area (RPA) after storage for 0-24 hours, respectively. The analytical results represent the method is accurate and has repeatability.

Total saponins

50.0 g powdered chilli fruits were Soxhlet extracted with methanol for 8 hours. The solution was distilled at 45°C under reduced pressure, and the resulting soft-mass was refluxed with ethyl acetate. Ethyl acetate insoluble fraction was dried in vacuum oven at 750 mm-Hg for 8 hours, at 60°C and weighted for total saponins content. The extract was dissolved in methanol and analysed using TLC/-HPTLC (Camag make, using winCATS System Manager Software and HPTLC Silica gel $60F_{254}$) with mobile phase chloroform: methanol: water (65: 35: 5). Spots were developed by anisaldehyde-sulfuric acid reagent (0.5 ml p-anisaldehyde in 50 ml glacial acetic acid and 1ml of 97% H_2SO_4) after heating at 90°C for 10 minutes. The chromo-plate was examined at visible light, and Rf values were calculated.

Chili oleoresin

The whole chili fruit dried, powdered and sieved through 20 mesh, 100 gm each, was extracted with 400 ml of ethanol at 60°C, each of three hours, at four times with stirring, using Remi Laboratory Stirrer (Cat no.; RQT-127A), filtered, and distilled-out at 60°C using rotary vacuum evaporator. The resulting oily mass was further dried in a vacuum dryer to remove traces of solvent at 60°C for 8 hours, at 750 mm-Hg, allowed to attain room temperature in a desiccators and yield was calculated.

Total anthocyanins

Total monomeric anthocyanins were determined using cyaniding-3-glucoside as a reference standard by the pH differential method [8]. Buffer, pH=1 (potassium chloride-hydrogen chloride) and pH= 4 (acetic acid-sodium acetate) were mixed with samples and absorbance were measured at 520 nm and 700 nm, respectively.

Total polyphenols

Total phenolics were calculated against gallic acid using the spectrophotometer, as per ISO 14502-1 [10]. In short sample solution (1.0 ml), in duplicate, was mixed with 5 ml of Folin-Ciocalteu's reagent (1/10 dilution in water) followed by sodium carbonate solution, 4 ml (7.5%, wt/ v), kept at room temperature for 60 min and absorbance was measured at 765nm.

Soil analysis

The soil samples were dried at ambient temperature, grounded and sieved using a 0.5 mm stainless sieve, stored at 5°C and analysed for the relevant properties using standard procedure [11]. The soil type was noted on the physical appearance and pH value using 1% solution in DM water.

Statistical analysis

Where applicable, results were expressed as a mean ± standard deviation. Calibration curves were plotted by linear regression analysis of absorbance of the compound used as a reference standard against its concentration. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Capsaicin and total capsaicinoids

Evaluation of the HPLC fingerprints of chilies from different locations shows the retention time of common peaks in each analysis was consistent with others (Fig. 2), although the relative peak area differed significantly. Evaluation of results showed the lowest pungency in Meitei Morok, followed by Morok and Yelhlang Morok and highest in the Naga Mircha /Umorok.

An attempt to use the pungent concentration as a taxonomic tool could not be possible to distinguish a species [12] though it is an aid to identifying chilies (Family; Solanaceae) fruits [13]. The pungency is an adaptive tool tightly linked to a protective role of frugivores and seed predators, which is produced during fruit maturation after elongation is complete [14]. These secondary compounds are a mediator in fruit-frugivores interactions as acts on individuals, between fruiting plants, their mutualists, predators and parasites, including seed predators, insects and microbial frugivores [15, 16]. Capsaicinoids are potent anti-microbial [17] though microbes are ubiquitous frugivores but detrimental to plant health and do not disperse seeds. Plant populations within their range adapt sufficiently with the existing selection pressure through abiotic or biotic habitat conditions [18]. Local adaptation leads to a home-site advantage, a higher fitness of resident plant species in comparison to introduced congeners. As per integrated community concept [19] the synergistic interactions and tolerances of species to abiotic conditions leads to allelopathy [20].

Nature has designed chili fruits to facilitate seed dispersal and impart features to increase attractiveness to avian capable of ingesting and defecating viable seeds after consuming fruits. In a study to differentiate the disperser rates of pungent and non-pungent fruits by seed-dispersing birds, there was no preference based on pungency. The chilli fruits once ingested, capsaicin affects the gut processing of seeds. Capsaicin increased seed retention times by an average of 15-20% compared with controls. Longer retention of seeds in gut increases assimilation of nutrients in fruit pulp as well as the dispersion to more remote places due to prolonged exposure to digestive enzymes and physical processing. Dispersed seeds at a remote location on germination has to adopt the local environmental conditions that may not be beneficial always [5]. These newly introduced populations have to interact with selection pressure leads to the genetic diversity due to the propagule pressure, in both the cases of natural and anthropogenic dispersal [21]. The new saplings at a distinct place may turn into genetic diversity under propagule pressure, using pungency as a defense tool, resulting in different shape, size and chemical composition. In the present study, the highest concentration of capsaicinoids in Naga Mircha / Umorok (*C. chinense*) from Saijang, District Peren and a limited presence of Yelhlang Morok to Bishnupur District may be a result of the propagule pressure.

Total anthocyanins

Anthocyanins impart additional attractive colors to chili fruits. In present study total monomeric anthocyanins (Table-1, Table-2), does not establish any promising co-relation among cultivars but re-confirms the facts for availability and its biological importance for attracting pollinators and preventing the photo-oxidative damage due to free radicals during metabolic processes [22]. These compounds have anti-inflammatory, anti-carcinogenic, cardiovascular disease prevention, obesity control and diabetes alleviation properties [23]. Chili anthocyanin biosynthesis and accumulation is a highly regulated and complex process [24]. Accumulation of anthocyanins is affected by several chemical and physical stresses [22] therefore the data concludes that the stresses responsible for anthocyanin biosynthesis and accumulation are almost same in the region of District Peren, where capsaicinoids concentration was reported highest in the present study.

Total phenolics

Total phenolics were estimated in different cultivars from the region (Table-1, Table-2), marks the traditional dietary value of these fruits as potent antioxidant, useful in the prevention of various oxidative stress associated diseases such as cancer [25]. Phenolics are known for managing non-toxic interactions with herbivores and symbiotes. Avian consuming chili fruits gain internal protection from phenolics and disperse the seeds, even in remote places with fecal material. There was no co-relation between the concentration of anthocyanins and total phenolics in the fruits (Fig. 3).

Saponins

The assay for total saponins was 5.43% ± 1.64 in all samples. The extract generates frothing on mixing with distilled water while on TLC/-HPTLC analysis a brownish band at Rf= 0.38 is common in all but highest concentration in Yelhlang Morok, followed by Morok and Umorok, least in Meitei Morok. Characteristic bands for Morok (S1) (Rf 0.25, 0.31and 0.85), Meitei Morok (S2) (Rf 0.27 and 0.85), Naga Mircha /Umorok (S4) (Rf. 0.29, 0.74 and 0.81) and Yelhlang Morok (S3) (Rf. 0.38) provides a comparative glance (Fig. 4) of cultivars for saponins. These fruits have many folds differences in market prices. The saponins fingerprints can be used to identify the powdered product in the market for authenticity of the cultivar. The possible adulteration and spurious preparations by using dye Sudan-1 to adulterants (mustard seed husks, sawdust, salt and bulked with ground rice) [26], do not have these characteristics. Saponins, an important part of plant defense system against microbial infection [27] in chili fruits have additional support for its therapeutic value to increase the body immunity.

Table-1: Compositional differences in chilies; *in-situ* collection, cultivars of Manipur and Nagaland, India

Accession No.	Name of Place	Coordinates	Altitude (m)	Species	Oleoresin (% w/w)	Pungent (% w/w)		Anthocyanins (mg/ kg)	Total phenolics (mg/ kg)
						Capsaicin	Capsaicinoids		
DDJ/002	Jalukia (B)	N 25°36/ E093°42/	491	*C. chinense*	27.62	1.12	1.80	9.74 ± 0.52	22,398 ± 215.7
DDJ/003	Old Jalukia	N25°34/ E093°43/	789	*C. chinense*	27.59	1.09	1.68	10.76 ± 0.35	23,481 ± 185.3
DDJ/004	Sirhima Village	N 25°44/ E 093°53/	399	*C. chinense*	27.61	1.11	1.73	8.06 ± 0.31	22,354 ± 174.9
DDJ/007	Midziphema	N 25°45/ E 093°51/	450	*C. chinense*	27.01	1.20	1.85	10.28 ± 0.67	21,626 ± 215.5
DDJ/009	Laungdi, (Tinening)	N 25°19/ E 093°35/	820	*C. chinense*	27.50	1.21	1.81	16.54 ± 1.32	23,923 ± 219.7
DDJ/010	Old Nazau, (Tinening)	N 25°17/ E 093°37/	1234	*C. chinense*	27.41	1.22	1.81	28.14 ± 3.25	37,638 ± 318.2
DDJ/011	TNU Jhum Field	N 25°14/ E093°36/	723	*C. chinense*	27.61	1.30	1.89	13.21 ± 0.98	24,646 ± 231.2
DDJ/012	TNU Jhum Field	N 25°15/ E093°36/	871	*C. chinense*	27.61	1.26	1.88	12.86 ± 1.23	24,483 ± 261.3
DDJ/013	Old Nazau (Tinening)	N 25°17/ E093°38/	1250	*C. chinense*	27.68	1.19	1.72	16.85 ± 2.14	28,534 ± 354.5
DDJ/014	Nkialwa, (Tinening)	N 25°25/ E093°38/	1581	*C. chinense*	27.81	0.96	1.54	18.15 ± 2.11	28,975 ± 317.2
DDJ/016	Uripok (Imphal W.)	N24°48/ E 093°56/	759	*C. frutescens* (Meitei Morok)	19.61	0.04	0.07	19.35 ± 1.84	23,619 ± 235.8
DDJ/017	Bishnupur	N 24°37/ E 093°45/	795	*C. frutescens* (YelhlangMorok)	23.72	0.43	0.90	20.67 ± 1.56	31,187 ± 365.8
DDJ/018	Nambol	N 24°43/ E 093°50/	734	*C.frutescens* (YelhlangMorok)	23.74	0.16	0.74	21.65 ± 1.52	34,123 ± 352.1
DDJ/019	Lamphel (Imphal W.)	N25°48/ E093°55/	776	*C. annuum*	17.31	0.06	0.09	18.16 ± 0.95	21,658 ± 204.3
DDJ/023	Nambol	N 24°42/ E 093°50/	786	*C. chinense*	27.62	1.08	1.76	26.25 ± 2.01	25,633 ± 215.2
DDJ/024	Sagolband (Imphal W.)	N24°48/ E093°56/	829	*C. frutescens* (Meitei Morok)	19.31	0.04	0.07	21.09 ± 1.03	25,865 ± 268.2
DDJ/025	Lamphel (Imphal W.)	N 24°48/ E 093°55/	789	*C. annuum*	17.30	0.06	0.09	19.46 ± 0.79	20,655 ± 169.5
DDJ/026	Hatta (Imphal W.)	N 24°48/ E 093°56/	788	*C. annuum*	17.28	0.06	0.09	21.13 ± 0.54	21,497 ± 212.2
DDJ/027	Bishnupur	N24°34/ E093°38/	857	*C. chinense*	27.61	1.23	2.07	23.46 ± 1.21	23,650 ± 285.6
DDJ/028	Athibung Town	N25°33/ E093°37/	650	*C. chinense*	27.63	2.05	3.14	25.14 ± 0.86	26.485 ± 216.4
DDJ/029	Athibung	N25°33/ E093°37/	650	*C. chinense*	27.60	2.02	3.17	26.15 ± 0.88	26,613 ± 261.2
DDJ/030	Saijang	N25°35/ E093°39/	445	*C. chinense*	26.94	2.09	3.24	24.62 ± 0.65	25,986 ± 256.8

Oleoresins

Chili oleoresin, the oily mass (Table-1, Table-2) contain pigments, predominantly capsanthin [28]. In the present study, Naga Mircha /Umorok were found having highest yield of oleoresin than Yelhlang Morok and Meitei Morok and least in Morok. The oleoresin having all the nutritional components of chilli fruit is used as a natural colorant in daily food and beverages, globally [29].

Table-2: Compositional differences in chilies; ex-situ sampling, cultivars of Manipur and Nagaland, India.

Accession No.	**Name of Place**	**Coordinates**	**Altitude (m)**	**Species**	**Oleoresin (% w/w)**	**Pungent (% w/w)**		**Anthocyanins (mg/ kg)**	**Total phenolics (mg/ kg)**
						Capsaicin	**Capsaicinoids**		
DDJ/001	Medziphema	N 25°45/ E 093°51/	454	*C. chinense*	27.8	1.01	1.79	9.51 ± 0.54	21,451 ± 152.2
DDJ/005	Dimapur Sabji Mandi	N 25°54/ E 093°44/	149	*C. chinense*	28.01	1.16	1.76	9.67 ± 0.39	25,468 ± 268.4
DDJ/006	Old Dimapur Market	N 25°54/ E 093°44/	149	*C. chinense*	26.79	1.15	1.78	9.54 ± 0.42	25,984 ± 197.6
DDJ/008	Piphema	N 25°45/ E 093°57/	910	*C. chinense*	27.51	1.16	1.71	11.18 ± 0.98	20,986 ± 265.3
DDJ/015	Piphema	N 25°45/ E093°57/	907	*C. chinense*	27.65	1.21	1.79	12.13 ± 0.86	21,463 ± 168.6
DDJ/020	Nagamappal Market	N 24°48/ E 093°56/	760	*C. annuum*	17.18	0.05	0.09	21.80 ± 0.89	21,967 ± 167.6
DDJ/021	Pauna Bazar (Imphal W.)	N 24°21/ E 093°57/	760	*C. annuum*	17.20	0.06	0.09	20.45 ± 0.45	21,195 ± 170.4
DDJ/022	Bishnupur Market	N 24°37/ E 093°45/	805	*C. chinense*	27.53	1.21	1.88	23.98 ± 1.58	24,034 ± 179.5
DDJ/031	Jaluki Bazar	N25°38/ E093°40/	336	*C. chinense*	26.86	2.18	3.19	24.12 ± 1.23	26.865 ± 298.7
DDJ/032	Nrailuang, Nzau	N25°17/ E093°38/	1148	*C. chinense*	27.65	1.30	1.94	31.52 ± 2.31	30,658 ± 306.2
DDJ/033	Nzau Nrailuang	N25°17/ E093°38/	1140	*C. chinense*	27.08	1.28	1.96	31.86 ± 2.69	31, 287 ± 296.3

Table-3: Physico-chemical properties of soils from *in-situ* sampling sites.

Accession No.	**Location**	N_{RA} (mg/ kg)	K_{RA} (mg/ kg)	P_{RA} (mg/ kg)	N_T %	K_T %	P_T %	**pH**	**Soil type**
DDJ/002	Jalukia (B)	21.62± 0.06	281.54± 0.03	10.23± 0.05	0.28± 0.01	2.64± 0.02	0.03± 0.001	6.58	Dark brown soil
DDJ/003	Old Jalukia	20.14± 0.02	284.31± 0.05	9.85± 0.04	0.29± 0.08	2.67± 0.01	0.09± 0.001	6.56	Dark brown soil
DDJ/004	Sirhima Village	26.45± 0.02	287.81± 0.03	13.01± 0.04	0.34± 0.09	2.81± 0.02	0.04± 0.002	6.67	Dark brown soil
DDJ/007	Midziphema	19.10± 0.01	215.21± 0.05	9.56± 0.05	0.26± 0.01	2.34± 0.02	0.06± 0.001	6.87	Dark brown soil
DDJ/009	Laungdi, (Tinening)	19.91± 0.03	275.59± 0.04	8.34± 0.04	0.32± 0.02	2.31± 0.03	0.11± 0.001	6.54	Dark brown soil
DDJ/010	Old Nazau, (Tinening)	21.65± 0.05	284.83± 0.03	8.78± 0.07	0.33± 0.03	2.36± 0.02	0.12± 0.001	6.53	Dark brown soil
DDJ/011	TNU Jhum Field	23.44± 0.04	291.61± 0.05	15.87± 0.07	0.36± 0.02	2.87± 0.01	0.14± 0.001	6.46	Dark brown soil
DDJ/012	TNU Jhum Field	23.21± 0.04	298.15± 0.05	13.54± 0.06	0.35± 0.03	2.69± 0.02	0.23± 0.002	6.65	Dark brown soil
DDJ/013	Old Nazau (Tinening)	24.04± 0.04	285.56± 0.06	12.98± 0.06	0.36± 0.03	2.81± 0.02	0.26± 0.002	6.68	Dark brown soil
DDJ/014	Nkialwa, (Tinening)	23.87± 0.03	307.18± 0.05	11.68± 0.05	0.35± 0.02	2.56± 0.03	0.15± 0.001	6.43	Dark brown soil
DDJ/016	Uripok (Imphal W.)	26.87± 0.07	249.87± 0.06	7.14± 0.03	0.31± 0.01	2.16± 0.02	0.05± 0.002	6.24	Black soil
DDJ/017	Bishnupur	26.87± 0.06	268.17± 0.03	8.96± 0.05	0.33± 0.02	2.27± 0.02	0.09± 0.002	6.48	Black soil
DDJ/018	Nambol	25.09±	261.12±	8.79±	0.32±	2.31±	0.12±	6.87	Black soil

		0.06	0.06	0.06	0.03	0.01	0.001		
DDJ/019	Lamphel (Imphal W.)	26.01± 0.05	245.23± 0.02	8.68± 0.03	0.32± 0.04	2.19± 0.01	0.11± 0.001	6.36	Black soil
DDJ/023	Nambol	26.71± 0.07	271.03± 0.03	7.98± 0.02	0.31± 0.01	2.30± 0.02	0.12± 0.002	6.23	Black soil
DDJ/024	Sagolband (Imphal W.)	26.15± 0.05	261.23± 0.07	8.02± 0.06	0.29± 0.02	2.18± 0.01	0.18± 0.001	6.29	Black soil
DDJ/025	Lamphel (Imphal W.)	24.68± 0.02	261.05± 0.03	8.13± 0.06	0.31± 0.03	2.21± 0.01	0.19± 0.001	6.28	Black soil
DDJ/026	Hatta (Imphal W.)	26.18± 0.06	260.10± 0.06	7.68± 0.04	0.31± 0.02	2.28± 0.02	0.12± 0.001	6.42	Black soil
DDJ/027	Bishnupur	24.05± 0.07	265.13± 0.02	8.13± 0.03	0.31± 0.03	2.32± 0.03	0.15± 0.002	6.18	Black soil
DDJ/028	Athibung Town	28.16± 0.09	402.11± 0.03	14.65± 0.06	0.42± 0.02	3.06± 0.01	0.51± 0.001	6.87	Dark brown soil
DDJ/029	Athibung	27.28± 0.05	381.56± 0.05	16.05± 0.05	0.41± 0.02	2.97± 0.02	0.49± 0.001	6.88	Dark brown soil
DDJ/030	Saijang	29.24± 0.08	302.41± 0.06	15.89± 0.04	0.451± 0.04	2.98±0.01	0.42± 0.001	6.85	Dark brown soil

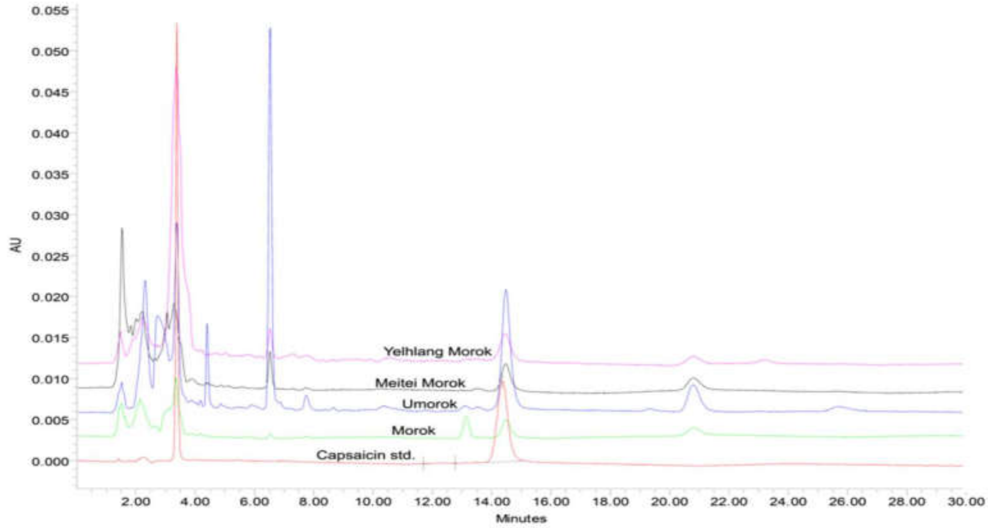

Fig. 2: Different chili fruits; overlay view of HPLC fingerprints.

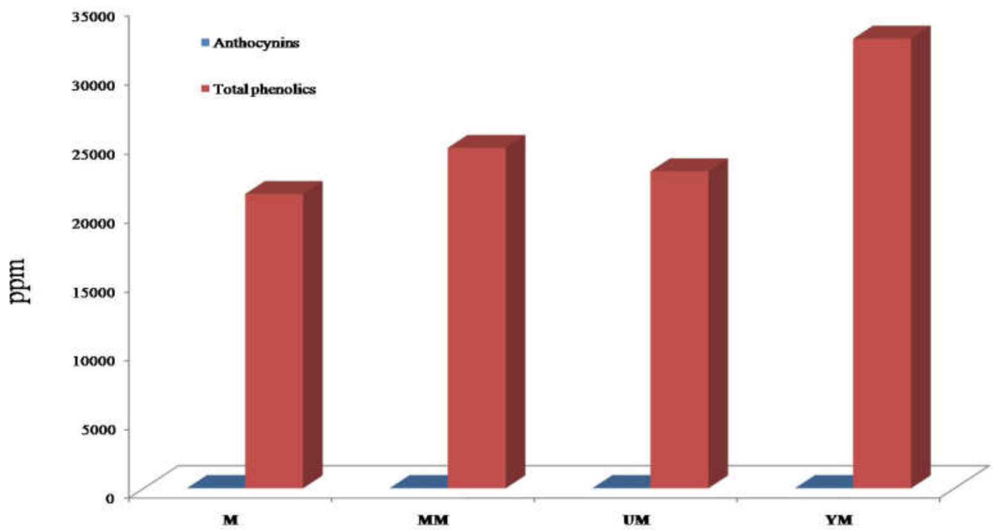

Fig. 3: Anthocynins and total phenolics in chili cultivers. M= Morok , MM= Meitei Morok, UM= Umorok, YM= Yelhang Morok

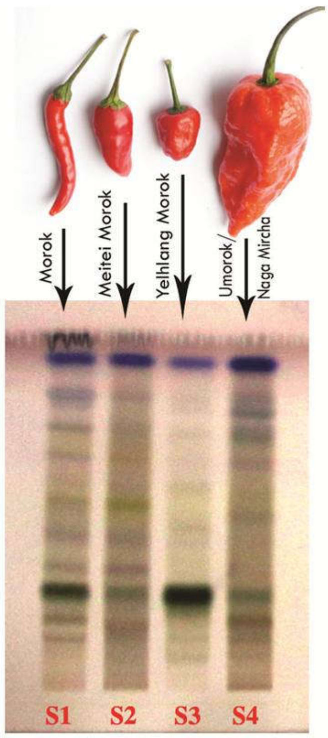

Fig. 4: Different chili fruits and saponins profile

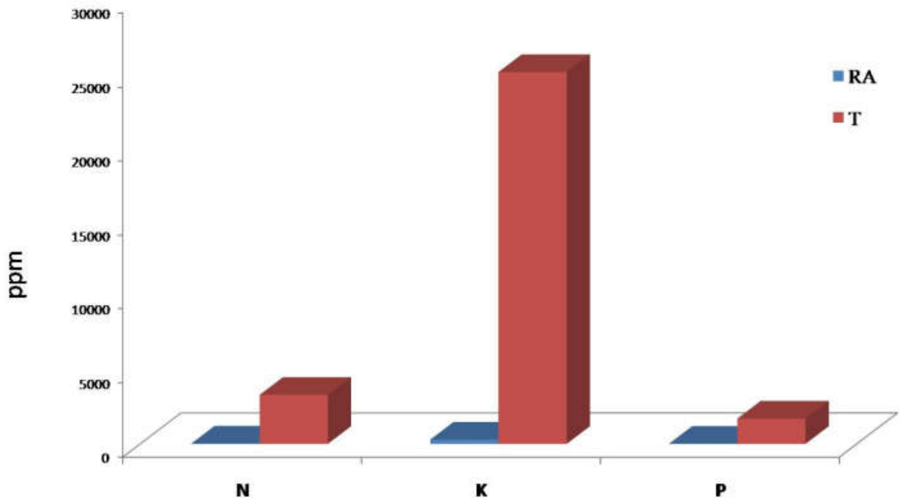

Fig. 5: Total (T) Minerals and rapidly available (RA) form.
N= Nitrogen, K= Potassium, P= Phosphorous,

Soil nutrients from the *in-situ* sites

Soil samples were analysed for rapidly available nitrogen (N_{RA}), rapidly available potassium (K_{RA}), rapidly available phosphorus (P_{RA}), total nitrogen (N_T), total potassium (K_T), total phosphorus (P_T) and pH value (Table-3). Soil structure affects the root growth and uptake of plant nutrient while the availability of nutrients affects the whole plant health, mainly depends on nitrogen (N), phosphorus (P) and potassium (K). Paddy and pulse cultivation and mulching their straw increases the biological nitrogen fixation process [30] in the soil and make a balance of nitrogen regardless of the type of soil. Paddy cultivation (in Jhums) and straw mulching after harvest (in valleys) increases the nitrogen content of the soil, both readily available and total nitrogen. Potassium increases crop yield and improves quality seeds. Its deficiency can cause reduced yield and quality. It has a vital role in enzymes activation for metabolic processes, especially the production of proteins and sugars though needs in small quantity [31]. Phosphorus enhances many aspects of physiology, including photosynthesis, root development, flowering, seed production and maturation, the deficiency can result in stunted, thin-stemmed and spindly plants [32]. Soil analytical results for essential element have no deficiency of these (Fig.5) and pH was between 6 and 7, though color varied from dark brown to deep black.

Oleoresins

Chili oleoresin, the oily mass (Table-1, Table-2) contain pigments, predominantly capsanthin [28]. In the present study, Naga Mircha /Umorok were found having highest yield of oleoresin than Yelhlang Morok and Meitei Morok and least in Morok. The oleoresin having all the nutritional components of chilli fruit is used as a natural colorant in daily food and beverages, globally [29].

3.6. Soil nutrients from the *in-situ* sites

Soil samples were analysed for rapidly available nitrogen (N_{RA}), rapidly available potassium (K_{RA}), rapidly available phosphorus (P_{RA}), total nitrogen (N_T), total potassium (K_T), total phosphorus (P_T) and pH value (Table-3). Soil structure affects the root growth and uptake of plant nutrient while the availability of nutrients affects the whole plant health, mainly depends on nitrogen (N), phosphorus (P) and potassium (K). Paddy and pulse cultivation and mulching their straw increases the biological nitrogen fixation process [30] in the soil and make a balance of nitrogen regardless of the type of soil. Paddy cultivation (in Jhums) and straw mulching after harvest (in valleys) increases the nitrogen content of the soil, both readily available and total nitrogen. Potassium increases crop yield and improves quality seeds. Its deficiency can cause reduced yield and quality. It has a vital role in enzymes activation for metabolic processes, especially the production of proteins and sugars though needs in small quantity [31]. Phosphorus enhances many aspects of physiology, including photosynthesis, root development, flowering, seed production, and maturation, the deficiency can result in stunted, thin-stemmed and spindly plants [32]. Soil analytical results for essential element have no deficiency of these (Fig.5) and pH was between 6 and 7, though color varied from dark brown to deep black.

CONCLUSION

Chili cultivars are maintained since centuries by Native population of the Northeast India, a reservoir of genes, have potential resistance to abiotic and biotic stresses. As the major geo-climatic conditions of the region remain more or less similar throughout the year, so topography, types of other vegetation and kind of soil do not seem to play much more on the hotness and types of chilies in the region. Besides the genetic difference and molecular engineering (hybridization followed by divergent or concerted evolution), climatic factors are major contributing factors in shaping the vegetation. The secondary biochemical in response to specific environmental stimuli, such as herbivore-induced damage, pathogen attack or nutrient deprivation increases the overall ability of plants to survive and overcome local challenges. These secondary compounds also manage inter-plant relationships acting as allelopathic defenders. The feeding deterrence by bitter, toxic and pungent is a potential tool of the plant for defense. However, equally plants have to foster many symbiotic relationships to survive, attracting the pollinators via colors and scent. The anthocyanins, capsaicinoids and polyphenols in chili fruits have the role of attractants, deterrents and nutrients, respectively, in the life-cycle of plant, and are the tool to increase the climatic tolerance. The co-existence of species in biodiversity hotspots experience increased selection pressure, resulted in reduced population due to the decay of dispersed seeds under high humidity and rich arthropods. These factors have led to the formation of nano hotspots as Bishnupur for Yelhlang Morok within the mega bio-diversity hotspot. A plant under adaptation to novel environmental conditions facilitate the geographical expansion of the species as has happened in the case of *C. annuum* from Brazil to Europe to Africa and Asia. Comparative chemical studies for the uniqueness of crops from small patches developed under propagule pressure on a particular site, the nano hotspots, lead to genetic diversity among cultivars, as in case of Yelhlang Mook in the present study. Each chili cultivar described in the study has immense socio-cultural importance, apart from being a vegetable and spice crop. Since hotness of chili is affected by several factors, Saijang (Nagaland) was found a location to having highest capsaicinoids in *C. chinense* in the region. Saijang area can be selected for commercial cultivation of the species in the region. The overall finding confirms the traditional chili cultivars as a potential source of therapeutic ingredients, though concentration varies with growing conditions of plant. Powdered fruits of these cultivars, the value added product, can be authenticated by chromatographic fingerprints of saponins. The commercial cultivation of these landraces is expected to gradually take-over; it is therefore timely to publish comparative parameters for sustainable trade of these natural resources.

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