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Dr. Kamlesh Kumar Yadav
Assistant Professor (Guest), Department of Botany, MLS College, Sarisabpahi, Madhubani, Bihar, India

Dr. Mahesh Prasad Yadav
Department of Zoology, BMA, College Baheri Darbhanga, Bihar, India

Corresponding Author:
Dr. Kamlesh Kumar Yadav
Assistant Professor (Guest), Dept. of Botany, MLS College, Sarisabpahi, Madhubani, Bihar, India

A study on pharmaceutical compounds in *Ocimum sanctum* L. (Lamiaceae) through genome doubling

Dr. Kamlesh Kumar Yadav and Dr. Mahesh Prasad Yadav

Abstract
Tulsi is the queen of herb, the legendary 'Incomparable one'. Its other name, VISHNUPRIYA means one that pleases Lord Vishnu. In English it is known as Holy BASIL and botanically called *Ocimum sanctum*. It is regarded as a kind of 'elixir of life' and believed to promote longevity as described in Ayurveda. The species is spread over parts of the globe. *Ocimum sanctum* has two varities that is black (Krishna Tulsi) and green tulsi (Rama Tulsi) but the chemical constituents are similar with common medicinal properties.

Keywords: *Ocimum sanctum*, phytochemical constituents, Lamiaceae

Introduction
Ocimum sanctum (small flower basil) belongs to the mint family (Lamiaceae). It is one of the major sources of commercially and medically important essential oils and contains many terpenes (i.e linalool) and phenols (i.e eugenol) that dominate this type of oil. The content of terpen and phenol in plants is used for various illnesses such as colds, headaches, inflammation, stomach upset, antidepressants, heart disease, fever, tuberculosis and various forms of malaria. The plants have been wonderful since ancient times. Giving importance, it is cultivated by all Hindu families in the courtyard. Leaf and inflorescence are the two main sources of the phytochemical constituents in Lamiaceae in general and the species *Ocimum sanctum* particular.
Considering the high medicinal and commercial importance of various terpenes and phenols of the species, its natural tetraploids with 2n= 4x=32 chromosomes, the basic chromosome number being x=8. It is, therefore, expected that *Ocimum sanctum* containing commercially valuable linalool and eugenol along with number of other aromatic terpenes and phenols, and the recent work carried out in this laboratory *Ocimum sanctum* an effort was made to develop useful autopolyploids with enhanced pharmaceutical chemical compounds.

Fig 1: Tetraploid Plant

Gnome doubling with the help of colchicine treatment was first reported by Blakeslee & Avery in 1937. Since then autopolyploid have been induced in a no. of plants. Autopolyploidy also increases gene expression on a per cell basis in proportion to the gene dosage conferred by the polyploidy level as demonstrated in a comparative study of monoploid (x), tetraploid (2x), triploid (3x) & tetraploid (4x) lines of maize (Guo *et al*. 1996).

Considering, therefore, expected that *Ocimum sanctum* containing valuable linalool & eugenol along with a no. of other aromatic terpenes & phenols, upon chromosome doubling, may give rise to autopolyploids having augmented contents of the pharmaceuticals compounds.

All the genera of this aromatic family in general & *Ocimum* in particular are of great medicinal & commercial importance. the species *O sanctum* contains a number of aromatic terpenes & phenols, namely, urosolic acid, methyl carvacrol, limatrol, caryophyllene, rosmarinic acid, estragol, eugenol (1-hydroxy -2-methoxy -4-allylbengene) (Sinha *et al*. 2007) [7] & linalool (3, 7 – dimethylocta–1, 6–dien -3.0l), (Sobti 1978, Gupta 1994).

The plant is natural tetraploid, with 2n = 4x = 32 chromosomes, & in principle is expected to contain double dose of genes for many of the characters. The progeny of polyploids is generally chromosomally unbalanced and genetically unstable, leading to abnormal segregation pattern and sterility problems. Alternatively, it is desirable to bypass sexual reproductive mechanisms and rely on asexual means of reproduction to capture vegetative parts, such as root, leaf inflorescence, etc. as a source of phytochemical principles (Lavania 2005).

Comparative study of Morpho Phenological, cytogenetic & phytochemical investigations were made in tetraploid source plant and its three autopolyploid generation.

Material and Methods

The herb understudy, that is, *Ocimum sanctum*, were collected from the various parts of the state and grown in the experimental gardens of the University Department of Biotechnology, Darbhanga. Seeds were collected from elite plant possessing good quality with regards to total biomass and disease resistance.

Selection of Elite Plants: The plant which is healthy and yields good herbage of phytochemical importance is known as elite plant.

Morpho Phenological Studies: Morpho Phenological studies like number of primary and secondary branches, no. and length of inflorescences per plant, fresh and dry weight of leaves and inflorescences and total biomass of the plants, were made on ten different populations collected areas of North Bihar, India.

A thorough Survey Was conducted to collect *Ocimum sanctum* from different localities of the state of Bihar, India. Various populations, thus collected, were grown in the Experimental Gardens, University Department of Biotechnology, Darbhanga, Bihar.

For the analysis of cytological characters several regents, Pre–treating mixture, Acetic Acid, Ethyl Alchol, Orcein etc. were prepared with different concentration. Mitotic and Meiotic studies were also done to prepare idiogram of chromosomes of tetraploid parent & its three autopolyploid generations.

Different concentrations of colchicine were made for the treatment of plant at different stages such as 4–6 leaved stage and 6–8 leaved stage at different intervals of time.

For Phytochemical Investigation, hydrodistilation / extraction of essential oil were done from fresh leaves and inflorescence from the elite (tetraploid) parent and synthetic autopolyploids plants with the help of Clevenger's apparatus. Different Chromatographs were made with the help of Gas Chromatography

Observation

Morpho Phenological characters were studied in tetraploid source plant.

Qualitative Studies of tetraploid Source plant: Qualitative characters like habit, habitat, root, stem, leaf, inflorescence, flower and its different parts, seeds and pollinating agents were studied thoroughly.

Quantitative Studies of tetraploid Source plant: Plants were divided into five groups each containing five plants for thorough study. Data were collected and noted properly. Characters like root area, shoot area, root- shoot ratio, no. and area of leaf, total no. of primary and secondary branches, fresh and dry weight of leaves and in inflorescence, length between whorls of flowers in an inflorescence, diameter of whorls, no. of whorls per inflorescence, length of upper and lower pair of stamens, length of carpel, diameter of pollen, pollen sterility in upper and lower pair of stamens, fruits having zero, one, two, three and four seeds, total no. of seeds and weight of hundred seeds.

Discussion

The species *Ocimum sanctum* of the aromatic mint family Lamiaceae is a natural Tetraploid 2n=4x=32 Chromosomes. (Table: 1)

Table 1: Quantitative Morpho Phenological characters of tetraploid (2n=4x=32 chromosomes): Vegetative

Character	Sample					MI ± SD
	1	2	3	4	5	
Area (cm^2)						
Root	I410.00	550.00	221.00	224.00	378.00	356.60 ± 138.46
Shoot	1208.00	1742.50	1677.00	1340.00	2400.00	1673.50 ± 463.81
Root / Shoot Ratio	1:4.69					
Height (cm) at						
1st Inflorescence	49.20	53.30	39.60	49.40	35.80	45.46 ± 7.39
1st Anthesis	57.50	61.50	58.70	39.70	60.50	55.58 ± 9.01
Total Number						
Prim. Branch	9.00	14.00	12.00	12.00	9.00	11.20 ± 2.17

Sec. Branch	27.00	29.00	28.00	31.00	26.00	28.20 ± 4.01
Leaf	1626.00	1559.00	1379.00	1701.00	1566.00	1566.20 ± 19.20
Inflorescence	71.00	42.00	81.00	88.00	57.00	67.80 ± 18.53
Leaf area (cm•)	2.20	2.20	2.00	2.00	2.00	2.08 ± 0.15
Fresh / Dry Weight (g)						
Root	4.00/1.70	4.00/1.50	3.00/1.40	2.00/0.85	4.00/1.60	3.40 ± 0.89/1.41 ± 0.33
Stem	65.00/11.00	31.00/7.00	22.00/4.00	21.00/4.00	41.00/7.50	36.00 318.11 / 6.70 ± 2.90
Leaf	35.00/7.00	26.0014.50	32.00/5.70	30.00/5.00	30.00/5.05	30.60 ± 3.28 / 5.45 ± 0.96
Inflorescence	12.00/2.00	13.65/3.00	11.70/0.50	11.80/1.00	13.85/0.89	12.60 ± 1.05 / 1.48 ± 1.01
Biomass (g)	15.65	16.25	13.35	14.65	15.30	15.04 ± 1.10

Prim. = Primary, Sec. = Secondary

It was envisaged that creation of autopolyploid from such a true natural Tetraploid plant would fetch significant results, as polyploidy is the most favoured cytological status in nature in Lamiaceae (Singh, 1995). Colchiploids were, therefore, synthesized in this medicinally and commercially important species. Relevant Morpho Phenological characters were studied in the tetraploid source plant and its autopolyploids.

Genome doubling through colchicine treatment led to the induction of autopolyploid in *Ocimum sanctum* during the present investigation. The medicinally and commercially important herb is a natural tetraploid (2n=4x= 32 chromosomes) and, as expected, showed regular bivalent formation followed by normal dis-juction 8:8 at anaphase I and no meiotic abnormalities could be detected.

The source elite plant of *Ocimum sanctum* and its three autopolyploid generations possessed some important terpenes and phenols in the essential oils of leaves and inflorescences. Of the various aromatic compounds detected with the help of GC in the leaf of elite tetraploid parent, three were alkenes (α-pinene, camphene and limonene), one alcohol (1, 8-cineole), one ester (geranyl acetate) and two phenols (eugenol and camphor). On the other hand, leaves of autopolyploid contained only two alkenes, that is, Co which showed the presence a third alkene, that is, β-caryophyllene. The latter could not be detected either in the tetraploid source or the rest of the two colchiploids generations. Except the Co leaves, an alcohol (1, 8-cineole) was found in both the C_1 and the C_2. The alkene, limonene present in the tetraploid, was absent from all the autopolyploid generations. On the other hand, β-caryphyllence, though absent from the tetraploid and the polyploids C_1 and C_2 leaves, was present in Co.

A comparison of oils of leaf and inflorescence or the three autopolyploids indicated that major components (geranyl acetate and camphor) decreased considerably, except α -pinene and camphene. Content of camphor in C_2 leaf rose to 80.69% from 62.47%, possibly due to shift of precursor and/or intermediates from inflorescence to leaf.

Leaf was considered an important source of aromatic compounds as in addition to camphor, it also possessed α-pinene, camphene and geranyl acetate in good harvestable strength. It might be mentioned that these three terpenes, too, are commercially very important and are in great demand in pharmaceuticals, cosmetics and perfumery market world.

Conclusion

The present work on the creation of autopolyploids in *Ocimum sanctum* L. yielded following significant results with respect to their morphophonology. Total biomass of the tetraploid plant was almost doubled in C_2 generation. Total length of inflorescence gradually declined in colchiploids. Length of rachis between two whorls decreased. As a result of decreased rachis length, total number of whorls per inflorescence fell. Diameter of mature whorl, though, remained the same. However, C_1 plants showed a higher value among the colchiploids, indicating an encouraging trend. Length of upper pair of stamen declined, while that of lower pair increased. In the tetraploid plant, reverse was the case. Carpel length, too gradually improved, finally reaching a length in C_2 that was higher than the tetraploid source plants. After perusal of all the morphological characters of the tetraploid parent and its autopolyploids, especially the C_2 ones, it was obvious that the latter underwent an overall improvement.

Chromosome doubling in *Ocimum sanctum* L. with the help of colchicine treatment led to the creation of genetically stable autopolyploids having augmented content of the medicinally valuable aromatic compound, the camphor. *Ocimum sanctum* is a natural tetraploid (2n=4x=32 chromosomes). Its autopolyploids (2n=4x=64 chromosomes) were genetically stable. The tetraploid parent contained α–pinene, camphene, limonene, geranyl acetate and camphor as the major components of essential oil from leaf. Inflorescence of the tetraploid and all its tetraploid had only three major components, namely, camphor, geranyl acetate and eugenol.

Chromosome doubling in *Ocimum sanctum* L. with the help of colchicine treatment, led to the creation of genetically stable autopolyploids having augmented content of the medicinally and commercially valuable aromatic compounds. With regards to phytochemical contents of the tetraploid and its induced autopolyploids.

Content of camphor –30.85% in the tetraploid and 38.42%, 28.66% and 17.07 in Co, C_1 and C_2 – far exceed the rest two available major compounds. Camphor was augmented to 80.69% of the total essential oil in the leaf of the autopolyploid. Such an increase in camphor (a phenol) content and decrease in terpenes were believed to due to shift of intermediate compounds from terpene biosynthesis to that of phenol. Hence, leaf was considered a far better source of camphor along with some other terpenes. Leaf area of autopolyploids showed considerable quantitative superiority over their tetraploid. In addition to the above major terpenes and phenols, leaf and inflorescence of the autopolyploids contained some minor compounds as well which were less than 1% of the total oil content.

Appearance and disappearance of some phytochemicals in the autopolyploids might be due to expression and suppression of the concerned gene/s in the new gene combination and novel cytogenetical environment emerged as a result of doubling of tetraploid gene dosage. Though, tetraploid leaf had some terpenes, autopolyploid leaf and

inflorescence showed little preference for the compounds. *Ocimu sanctum* contained bicyclic monoterpenes and sesquiterpenes compared to simple monoterpenes (acyclic and monocyclic ones) as observed in the members of Lamiaceae in general and other species of *Ocimum* in particular. Due to very high content of camphor (80.69%) in autopolyploid of *Ocimu sanctum*, it was recommended for Medicinal & pharmaceutical exploitation.

From the above comparisons and correlations discussed among the various characters of the induced autopolyploids, third generation of autopolyploids (C_2) of the camphor type of *Ocimum sanctum* possessed enhanced pharmaceutical compounds with superior and novel qualities compared to the tetraploid parent.

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