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Recent Advances in Basil Improvement

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The aromatic plants belonging to genus *Ocimum* is popularly known by the name Basil. Basil was derived from greek word "Basilica" which means royal plant. Among the *Ocimum* genus *Ocimum basilicum* is variously known as Sweet basil, French basil or Common basil. *Ocimum sanctum* is known as Sacred basil or Holy basil which is a very sacred plant according to Hindu belief. This herb is popular in traditional medicine as "The Queen of Herbs," "The Incomparable One," and "The Mother Medicine of Nature". These aromatic plants are native to Indian subcontinent and cultivated throughout Southeast Asian tropics (Makri & Kintzios, 2008).

Characteristics of the plant

Holy basil is a biennial or triennial shrub. The leaves of this plant on steam distillation yield a bright yellow colour volatile oil possessing a pleasant odour with an appreciable note of clove oil. The plant contains mainly phenols, aldehydes, tannins, saponin and fats. The essential oil components are eugenol (71%), eugenol methyl ether (20%), carvacrol (3%) and minor portions of nerol, caryophyllene, selinene, α-pinene, β-pinene, camphor, cineole, linalool etc. The plant is used as a pot herb; leaves are used as condiment in salads and other foods.

O. sanctum is an erect, herbaceous, much-branched, softly hairy biennial or triennial, which grows to a height of 30-75 cm. Leaves are entire, serrate, pubescent on both sides, flowers purplish or crimson, in racemes, fruits are sub-globose or broadly ellipsoid, slightly compressed, nearly smooth, pale brown or reddish with small black markings. Sabja seeds also known as Basil seeds or Tukmaria or falooda seeds. Basil seed is commonly used fresh in cooked recipes. It is also used for its medicinal properties in Ayurvedic medicine.

Researches for Basil Improvement

1. Induction of polyploidy in basil (*Ocimum basilicum* L.)

Production of an autotetraploid population of basil (*Ocimum basilicum*) by colchicine, different concentrations (0.00, 0.05, 0.10, 0.20, 0.50 and 0.75%) and four treatment methods were examined (seed, emergence of cotyledone leaves stage and emergence of true two type leaves stage, and root treatment) to determine the best treatment for the induction of tetraploid plants. Autotetraploid plants were produced only by treatment of growing point of seedlings, at the emergence of cotyledone leaves stage, and treatment with 0.5% proved to be the most effective in producing autotetraploids. The induced tetraploids in basil was accompanied by larger stomata and pollen grains, increase in chloroplast number in guard cells and decrease in stomata density, compared to diploid control plants. In order to distinguish the induced colchicine tetraploid plants from the diploids, morphological changes and techniques as stomata size, number of chloroplasts per guard cell, pollen grain diameter and flow cytometry were considered and proved that these methods are suitable, quick and easy methods for identification the ploidy level of *Ocimum basilicum* in various stages of the plant development of these species and among this methods flow cytometry as found to be the most efficient method for detecting induced changes in ploidy level (Omidbaigia *et al.*, 2010).

2. Genome sequencing, assembly and validation of holy basil

A whole-genome shotgun sequencing strategy by generating long and short paired-end reads, and mate-pair libraries was applied to assemble the 386 Mb genome sequence and the plastid genome of 142,245 bp of *O. sanctum*. That will help to understand its metabolic potential, diversity, regulation and evolutionary implications. SSR markers and protein profiling give the phylogenetic relationship with known species. Gene model prediction revealed the similarity of *O. sanctum* genome to *Nicotiana tabacum* and *Solanum lycopersicum*, all sharing same sub-class. GC content is an important indicator of the genomic composition, gene structure, intron size and number, gene regulation and stability of DNA. Average GC content of *O. sanctum* was 38.37%. Intron sizes in the genes of *O. sanctum* ranged from 5 to 8000 bp. Intron size variation among organisms may be due to inherent mutational processes generating insertions and deletions (Rastogi *et al.*, 2015).

3. Genetic variation in *Ocimum* species using RAPD and ISSR markers

There is lack information on the molecular characterization of *Ocimum* species. Efforts have been made under to characterize 17 *Ocimum* genotypes belonging to 5 different species (*O. basilicum, O. americanum, O. sanctum, O. gratissimum* and *O. polystachyon*) through RAPD and ISSR. PCR amplification using 20 RAPD primers generated a total of 506 loci, of which 490 (96.47%) loci were found polymorphic. The ISSR primers generated a total of 238 loci, of them 234 (98.17%) loci were polymorphic. Dendrogram was generated using the pooled RAPD and ISSR data showed all *Ocimum* genotypes in their respective species groups at a cutoff value of 0.49 and 0.42, respectively. Many unique species specific alleles were amplified by RAPD and ISSR markers. In both marker systems, a maximum number of unique alleles were observed in *O. sanctum*. Unique alleles can be converted in SCAR to develop species-specific diagnostic markers. The results provided valid guidelines for collection, conservation and characterization of *Ocimum* genetic resources (Patel *et al.*, 2015).

4. New variety of Tulsi more fragrant

Central Institute of Medicinal and Aromatic Plants (CIMAP) has successfully produced many Tulsi varieties with different medicinal and aromatic use. The new variety of Tulsi will have a higher aromatic compound compared to other varieties. It will provide aromatic compound required by perfume, incense and aroma industries in abundance within a short duration. The variety is used for essential oils with lemon scent. Kushmohak, a tulsi variety with smell of Khus grass used in essential oils with Khus grass fragance, CIM-Ayu has 83% of Eugenol which is usually extracted from cloves and has high extraction cost as it's not available in bulk and Vikarsudha tulsi with an earthy smell, a variety of purple colour with medicinal use CIM-Angna and CIM-kanchan and Somya has variant properties in terms of aroma. CIM-Jyoti, a variety in which scent of lemon and efficacy of Tulsi have been clubbed together in a single variety. The variety is used for essential oils with lemon scent. For cosmetic purpose the institute came up with CIM-Sharada rich in Methylchavicol could be used in vast range of beauty products for skin.

5. US scientists genetically engineering tulsi

A team of scientists at a US university are genetically engineering tulsi or basil to enhance its pharmaceutical value. Chandrakanth Emani, Assistant Professor of Plant Molecular Biology at the Western Kentucky University and his students are genetically engineering the basil to produce more eugenol, a compound in basil that has a very great pharmaceutical value because it's shown to control breast cancer. When you grind these basil leaves there is a compound called eugenol that comes out. Eugenol, when they put it on a plate where there are tumor cells, it stopped growth of the tumour cells. That was a proof of concept experiment which was done a long time back. If higher amounts of eugenol is present in basil plant, will be a storehouse of that anticancerous compound. The next phase in the research project would be to test the compound as an effective cancer treatment.

6. UV-B radiation to increase volatile oils in basil (*Ocimum basilicum* L.)

Exposure of *Ocimum basilicum* in a controlled environmental room temperature to supplementary UV- B light for 3 h per day in the early morning over a period of two weeks resulted in shorter plants with higher dry matter, thicker leaves and more axillary shoots. Supplementary UV-B did not affect plant leaf area or number of leaf- pairs; however, specific leaf area was significantly increased due to increase in leaf thickness. Analysis of volatile oils by TD-GC/MS in fresh leaf samples harvested after one and two weeks of treatment showed that UV-B also stimulated the synthesis of the phenyl-propanoid, eugenol and the terpenoids 1, 8-cineole and linalool. Overall, supplementary UV-B light effectively increased the total volatile oil content of basil leaves. There was no effect on volatile oil composition (Austen *et al.*, 2015).

7. Micropropagation

Basil micropropagation is usually achieved by axillary shoot proliferation from node explants. Solid culture media commonly consist of MS salts, 3% (w/v) sucrose and benzyl adenine (BA) alone or with gibberellic acid (GA3) as growth regulators. Rooting of the adventitious shoots takes place in half-strength MS medium supplement with BA and indolebutyric acid (IBA). A process for the multiplication of basil seedlings involving induction of multiple buds from apical, lateral or adventitious buds from basil explants. Bud induction, multiplication and regeneration culture media contained different concentration level of cytokinin and auxin. A stable production of basil plants of the same quality and with the high essential oil content was achieved quickly (Singh and Sehgal, 1999).

8. Somatic embryogenesis

Gopi and Ponmurugan recently described a protocol for plant regeneration *via* somatic embryogenesis from leaf explants of *O. basilicum*. Globular embryos were induced on MS medium supplemented with 2,4-D ($1mgl^{-1}$) and BA (0.5 $mg1^{-1}$) and further embryo development and maturation took place on MS medium supplemented with NAA (1 $mg1^{-1}$), BA (1 $mg1^{-1}$) and Kn (0.5 $mg1^{-1}$). The maximum induction rate was 75%, corresponding to 36.5 somatic embryos /culture (Mathew & Sankar, 2011).

9. Protoplast isolation and culture

Method for basil protoplast culture was involved culture of basil protoplasts in the presence of a basil cell suspension, resulting in efficient colony formation. Sweet basil leaves were cultured on solid Linsmaier-Skoog (LS) culture medium to produce callus culture, and calli were subcultured in LS liquid medium weekly to produce a suspension cell culture. The suspension was treated with enzyme solution containing 1% cellulase, 0.5% pectinase, 0.1% Pectolyase, 26 °C for 2 h. Protoplasts were obtained by filtration and centrifugation, followed by suspension in Nagata-Takebe (NT) medium, cultured at 26 °C for 25 days under 100 lux light. The colony formation rate was 19.6%, compared with 0.1% for a single culture control (Kholgade *et al.*, 2013).

10. *In vitro* production of secondary metabolites

Five clones of *O. basilicum* hairy roots, A-1 and A-2 and J-1, J-2 and J-3 were culture in three plant growth regulator-free liquid media (MS) containing 30 $g1^{-1}$ sucrose, Gamborg-B5 (B5) containing 20 $g1^{-1}$ sucrose and woody plant (WP) media containing 20 $g1^{-1}$ sucrose. After inoculation, all clones had rapid proliferation at the early stages of culture particularly in the WP medium. The highest amount of biomass recorded for any culture was 702.8 mg dry weight/flask for A-2 grown in MS medium for six weeks. Although in all media the hairy root cultures produced substantial amounts of rosmarinic acid (RA), particularly high levels were observed in MS medium (14.1% for J-1 at wk 8) and B5 medium (14.0% for A-2 at wk 6). These levels were almost 3.5-fold higher than those of an intact plant. The maximum yield of RA in all cultures examined was 73.5 mg/flask produced by J-1 in MS medium at wk 5. Small amounts of the related phenolics, lithospermic acid, and lithospermic acid-B were also obtained (Tada *et al.*, 1996).

Production of essential oil was carried out from 24-month old callus tissues of *O. basilicum*. The cultures were grown as static and in suspension cultures for the essential oil production. Both cultures were maintained by frequent subculturing every 4-6 weeks interval in fresh RT medium. The tissues were kept in the dark and cultivated at 26 °C with 55% of relative humidity. The maximum oil yield with dark-grown and light grown static cultures was 0.035 and 0.043 % of fresh weight, respectively. The cultures grown under illumination showed a significant increase in the constituent level, particularly linalool, and total oil yield (Purohit and Khanna, 1983).

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