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NEED OF BIOTECHNOLOGICAL STRATEGIES TO ENHANCE THE QUALITY AND QUANTITY PRODUCTION OF *Nelumbo nucifera*- AN ORNAMENTAL PLANT WITH HIGH NUTRITIVE AND THERAPEUTIC VALUE

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ABSTRACT

Lotus is an aquatic, perennial plant native to India having important medicinal, nutritional and ornamental values. Lotus extracts obtained from several different parts of the plant (lotus leaves, flowers, pods, roots/ rhizomes etc.) have been found to show a number of biological activities. A number of active compounds that are extracted from lotus can directly produce an effect in the medicinal field. Several studies and experiments are being conducted on lotus to map out the herb at a genetic level. Having some unique properties, lotus can easily attract researchers from the scientific community to ease the understanding of its different traits. This article aims to systematically review the need of different biotechnological strategies and techniques to improve and enhance the therapeutic and nutritional value of lotus along with the importance of the latest biological advancements and the ongoing studies and experiments that are being performed on the herb to enhance its quality and quantity of production.

Keywords: *Nelumbo nucifera*; nutrition; therapeutics; biotechnology; strategies.

INTRODUCTION

Lotus is an aquatic perennial plant belonging to the family *Nelumbonaceae*, consisting of one genus: Nelumbo and two species: *Nelumbo lutea* Pear. (popularly known as 'American Lotus') and *Nelumbo nucifera* Gaertn (famous as 'Asian Lotus') *Nelumbo nucifera* and *Nelumbo lutea* show major differences in the plant size, petal shape and colour, leaf shape and other external morphologies though the chromosome number (2n=16) is same for both. *Nelumbo nucifera* is also popularly known as 'sacred lotus' because of its religious importance in Hinduism, Buddhism, traditional Chinese and Indian cultures [1]. The distribution of lotus extends from northern (at an approximate range of 4593 feet to 4,600 feet in the southern Himalayan ends) and Central India, throughout Eastern Asia (northern parts and north to the Amur region; even the population of Russia has been referred to as "Nelumbo komarovii" at some point) and northern Indo-China with some aloof regions at the Caspian Sea [2,3]. It is an agriculturally important aquatic perennial herb with important ornamental and lucrative value and it is also an excellent source of food and nutrition [4]. Different parts of lotus, including its stems, seeds, flowers, leaves, pods etc. are consumed as different forms of food- the soft and delicate stems, rhizomes and leaves of lotus are rich sources of proteins and minerals, the seeds of lotus can be used as the substitutes of

coffee and when prepared into powered forms these can be used to prepare breads, the stamens and the flowers can be used for flavoring teas and beverages [5]. Besides this, lotus is also consumed whole as a vegetable in certain places. A number of food items (including various herbal teas, beverages, soups, curry items and others) can be processed and prepared from these different parts of lotus. Thus lotus can be considered an important option and source of several food nutrients in the present day world [6,7]. Besides its food value, lotus is also considered an important medicinal herb. Different parts of lotus can produce different biological effects and can be effectively used in the treatment and prevention of a number of diseases and complications [8]. Also, the secondary metabolites and extracts produced from different lotus parts can be used for a number of purposes. They can display anti-inflammatory, anti-oxidant, anti-rhythmic and several other effects [9]. In the present day world, lotus is considered an important horticultural herb; a number of studies and experiments are being conducted on lotus and with these ongoing works, the overall economic importance of lotus can be enhanced [4,10,11]. Application of different biotechnological and bioengineering strategies to detect and control early and later flowering of lotus, increasing the size of lotus rhizome and seeds, competitive analysis between different samples of lotus to identify the reasons of variation in different aspect of the herb (its shape, size, colouration, distribution, *in vitro* generation and others) are the common most studies that have been conducted on lotus [12-16]. With the use of these strategies, the nutritional profile and therapeutic index of lotus can be enhanced, since improvement in the quality and quantity of lotus production will directly and significantly affect the overall (medicinal, nutritional, ornamental, remunerative and others) economic profile of lotus [4,11]. It is the national flower of Vietnam and India. It is also found in Sri Lanka, New Guinea, Eastern and Northern Australia, Southern India. Specifically for *Nelumbo lutea,* the distribution extends throughout the northern parts of South America and the southern and eastern parts of North America; whereas *Nelumbo nucifera* can be found towards the north of Oceania and throughout Asia [17,18].

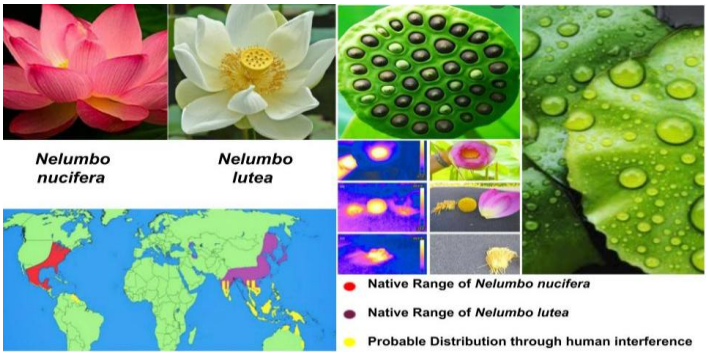

Fig. 1. Species distribution of lotus (a) *Nelumbo nucifera* Gaertn and *Nelumbo lutea* Pear (b) Seed Longevity, Thermal Imaging, Water-resistant Lotus Leaves displaying the Ultra Hydrophobicity property (c) Worldwide distribution of the two lotus species

Lotuses are commonly cultivated in the water gardens Fig. 1 shows the species distribution of lotus Two species of the *Nelumbonaceae* family- *Nelumbo nucifera* Gaertn, the Asian Lotus and *Nelumbo lutea* Pear, the American Lotus. The worldwide distribution of Lotus is also displayed; the red area covers the native distribution of *Nelumbo lutea,* the area in violet displays the native range of *Nelumbo nucifera* and the yellow area covers the probable distribution and extension of both the species of lotus herb through human interference. Seed longevity, a miraculous and unique feature of Lotus Seeds; Thermal Imaging showing the Floral Thermoregulation property of Lotus flower; Water-resistant lotus leaves covered with dense layers of waxy papillae and displaying the Ultra Hydrophobicity property of lotus leaves, are unique characteristic properties of the herb. This beautiful ornamental herb with high therapeutic and nutritive value is an interesting point of interest to the scientists. Analyzing study reports of different strategies utilized by a number of biological researchers, our review article aims to highlight the importance of biotechnology in enhancing the overall value of lotus.

MATERIALS AND METHODS

A number of reports, works and findings of different scientists have been studied in this manuscript. Various methods and biotechnological strategies have been scrutinized and thoroughly analyzed.

Distinctive Properties of Lotus

Lotus can be easily distinguished from other plant species because of its certain unique and important properties. Features like floral thermoregulation, leaf ultra hydrophobicity and seed longevity are unique to lotus (Fig. 1). The fruit of lotus is popular for longevity. It has been discovered that the fruits of lotus buried deep underground can still be germinated even after 1300 long years. Understanding this underlying procedure of lotus fruit longevity will contribute towards the improvement and enhancement of the storage of lotus seeds for a quality amount of time, thus leading towards certain appreciable uses in the field of agriculture and healthcare [19]. Studies have indicated that the primary factor resulting in this unique feature in lotus is probably due to the chemical composition of the fruit wall of lotus containing huge quantities of mannose, galactose, and tannins [20]. Simultaneously it was also found that the content of polyphenol in the epicarp of lotus seed keeps increasing with its ripening and displays a very strong antioxidant activity [21]. In case of the lotus leaves, the “lotus effect” or leaf ultra hydrophobicity is an important phenomenon [22]. This unique feature in lotus ensures that the upper epidermis of lotus leaves do not stay covered with water so that they can properly function with their normal activities and the stomataass can function properly [23]. Ultra hydrophobicity of lotus leaf is considered to be an advantage in its evolution procedure. According to studies, a special clump or layer of waxy papillae is responsible for this effect [24,25]. *NnCER2* and *NnCER2-LIKE* are the two important genes related with the wax biosynthesis of lotus [26]. Another feature, the floral organ thermogenesis is unique to lotus and takes place at the receptacle, stamen and petal of the lotus, respectively [27]. This unique feature can attract insects and pollinators enhancing the sexual reproduction of the herb [28]. The heat which is generated provides a warm and soothing environment for the pollinators and simultaneously releases different volatile compounds for attracting beetles and insects [29,30].

Various features and properties of lotus have stirred the scientific community, who aimed to explore lotus at different levels.

Lotus as a Source of Nutrients and Therapeutics

Because of its importance as a medicinal, ornamental and nutritional herb, the sacred lotus has gained much popularity in different scientific fields. A number of different food items and beverages can be prepared from different tissues and parts of lotus. A good quantity of nutrition can be obtained from the consumption of lotus parts. Also, because of being a traditional herb, lotus is extensively used in the preparation of different herbal and ayurvedic products. Different parts of the lotus have different important uses.

Roots/Rhizomes of Lotus as a Source of Food and Nutrition

The rhizomes of lotus can be consumed in different forms- raw, roasted, stewed, fried, pickled or cooked and it is also used in the preparation of herbal tea and other beverages [6]. It contains about 9.7% of carbohydrate, 1.7% of protein and 0.1% of fat and 1.1% of ash [31]. It can be used in the treatment of a number of diseases including dysentery, piles, dyspepsia, pharyngitis. Extract obtained from rhizomes can be used to treat diabetes, hypoglycemia and obesity [32]. It has also been mainly found to possess antioxidant, diuretic and antipyretic activities [9]. Lotus rhizomes also contain high amounts of dietary fiber, different vitamins, minerals, digestible starches [33]. A number of extracts and alkaloids can be obtained from lotus rhizomes which include ascorbic acid, riboflavin, thiamine, betulinic acid and niacin. The rhizomes have been further found to possess psychopharmacological, anti-microbial, anti-oxidant, hypoglycemic, diuretic, anti-inflammatory, antipyretic, antidiarrheal properties [9]. Liensinine, extracted from lotus rhizome, can effectively treat arrhythmia [34].

Seeds of Lotus as a Source of Food and Nutrition

The seeds of lotus can be consumed in raw, roasted or cooked forms. In countries like China and Japan, the seeds are often cooked and prepared into a number of traditional soups, pastries and desserts. It has also been found that lotus seeds possess phenolics, saponins and carbohydrates in good quantities [34]. According to the nutritional aspect, lotus seeds contain 10-16% of protein, high amounts of carbohydrate accounting to about 70-73%, 2-3% fat, 2.7% of crude fiber, 10-11% of moisture, and about 348.45cal/100gm. The seeds are also full of appreciable quantities of different minerals including copper, zinc, iron, manganese, magnesium, sodium, chromium, etc. As reported by some researchers [35]. Lotus seed cotyledons possess 3.9% of tannin and about 4% of phenolic content. The seeds are a good substitute for caffeine and are often consumed in coffee form [6]. Flour prepared from these seeds have been reported to have a 19.50mg/g polyunsaturated fatty acid lipid content where palmitic acid has been found to be the most available and abundant saturated acid and linoleic acid being the most available unsaturated fatty acid [33,11]. It was noted that the ethanolic seed extracts of lotus lead to a significant amount of increase in the differential and total lymphocyte and leukocyte population in treated rodents and mice, thereby providing an indication of the immunological effect of lotus seeds [2]. The seed extracts are also very much effective in treating foot paw edema [33]. Neutrophil

adhesion was not found to be majorly altered (like that of lotus rhizome extract treatment) when treated with lotus seed extracts. The seeds are used in the treatment of high blood pressure, vomiting, coughing, fever, chronic diarrhea, leprosy and also as an antidote to poison [32]. The seeds can be used to boost hormonal activities and proper hormonal functioning of the body. Lotus seeds and seed extracts have been found to possess anti-inflammatory, anti-depressant, anti-fibrosis, anti-proliferative, anti-viral, anti-obesity, hepatoprotective, immunomodulatory, free radical scavenging, astringent, hypolipidemic activities [9]. An extract obtained from lotus seeds, (S)-armepavine, displays immunomodulatory effects in mice, thereby increasing their rates of survival after treatment [36]. D-galactose, D-mannose, D-glucose and L-arabinose are the major seed polysaccharide contents of lotus [37]. Along with that, a good number of different extracts and alkaloids like lotusine, isoliensinine, liensinine, pronuciferine, nuciferine, neferine, dauricine, armepavine, roemerine, procyanidin, saponins, isoquininolinol can be obtained from lotus seeds [8].

Lotus Flower as a Source of Food and Nutrition

The petals of lotus flower are used in the preparation of some beverages and herbal tea and flavoring them [6]. Lotus flower can be used in the treatment of fever, liver diseases, cholera, heart palpitations, dysentery, vomiting, diarrhea, strangury and is also consumed for treating premature ejaculation [8]. It is often used as a tonic for the skin, liver and heart. The flower and its extracts have been found to contain antioxidant, antimicrobial, aphrodisiac, vasodilating, antiarrhythmic, antihypertensive, antipyretic, hypoglycemic, hepatoprotective, aldose reductase inhibition properties [9]. The flowers are also used as sedative, refrigerating and cooling agents. A number of extracts and alkaloids including nelumboroside A and B, glucopyranoside, kaempferol, quercetin, isorhamnetin, myercetin, arbutin, myo-inositol, adenine, B-sitosterol can be obtained and isolated from lotus [32]. Lotus flower contains a good amount of calcium, phosphorus, proteins, minerals, vitamin B1, vitamin B7, vitamin C, carotene, nicotinic acid, fat, fiber, iron [6]. It is also often used for the treatment of nose bleeding, hypertension, swelling, irritation and also used for treating insomnia. Fiber obtained from lotus flowers is consumed for maintaining diet and weight [32].

Lotus Leaves as a Source of Food and Nutrition

Lotus leaves have been found to possess astringent and diuretic properties and can be helpful in treating hypertension, diarrhea, strangury, fever, sweating [6]. The leaves can be grinded into a fine paste and applied on the body during high fever, it provides relief. The leaves and leaf extracts can treat and ease the complications of diabetics and show inhibitory activities against complex diabetic factors [38-41]. Lotus leaves can also be used to treat skin inflammations and rashes. In traditional therapeutics and common medicine, lotus leaves are vividly used for treating heat imbalance in the body and high blood pressure. Leaves of lotus can also be helpful in treating a number of diseases including different cardiovascular complications, cholesterol problems, obesity, etc. Hyperlipidemia can be effectively treated with lotus leaves extracts and also the level of lipid in the body can be well maintained and controlled at the same time with the use of lotus leaves [42]. Thus in traditional Chinese medicine, lotus leaves are often

used to prepare a weight-loss herbal tea. It has also been reported that the extracts of lotus leaves are effective in treating obesity in mice and rodents [43,44] and helps in lowering high cholesterol levels in them along with keeping in check the levels of triglycerides and phospholipids. Various abnormal bleeding disorders like epistaxis, hematemesis, hemoptysis, metrorrhagia, hematuria can also be effectively treated with lotus leaves [45]. It has also been reported by various scientists that lotus leaves and its extracts possess a radical scavenging ability along with a very strong antioxidant property. From the ethanolic extracts of lotus leaves, two anti-HIV principles could be isolated [46]. A number of important extracts and alkaloids can be isolated from lotus leaves-nuciferine, pronuciferine, N-nornuciferine, norcoclaurine, negferine, nelumboroside, anonaine, roemerine, coclaurine, remerine, liensinines, armepavine, asimilobine, quercetin, leucodelphinidin, leucoantho-cyanidin, etc. N-nornuciferine and nuciferine have been reported to be the two most important bioactive alkaloids present in the lotus leaves [47,32,48].

Medicinal / Biological Effects Produced by Different Parts of Lotus

Different parts of lotus herb including its rhizome, seeds, leaves, flowers can produce a number of biological and medicinal effects. It is often used in Chinese and traditional medicines. Table 1 lists the therapeutic and medicinal effects produced by lotus parts.

Table 1. Biological and therapeutic activities produced by lotus rhizome, seeds, leaves and flowers

Part of Lotus	Effects
Rhizome	Arrhythmic, psychopharmacological, anti-microbial, anti-oxidant, hypoglycemic, diuretic, anti-inflammatory, antipyretic, antidiarrheal
Seeds	Anti-inflammatory, arrhythmic, anti-depressant, anti-fibrosis, anti-proliferative, anti-viral, anti-obesity, hepatoprotective, immunomodulatory, astringent, hypolipidemic, anti-ischaemic, anti-fertility, anti-oxidative.
Leaves	Antioxidative, anti-viral, anti-inflammatory, anti-obesity, cardiovascular, lipolytic, hypocholesterolemic.
Flower	Antioxidative, antimicrobial, aphrodisiac, vasodilating, antiarrhythmic, antihypertensive, antipyretic, hypoglycemic, hepatoprotective, aldose reductase inhibition.

EXPERIMENTAL STUDIES PERFORMED TO ANALYZE CERTAIN BIOLOGICAL ACTIVITIES DISPLAYED BY LOTUS EXTRACTS

Many studies have been conducted on lotus till date to identify the biological effects and activities of its different parts (rhizome, leaves, flowers, seeds). In this aspect, a comparative study conducted by a group of researchers [49] is worthy of mention. They analyzed the antioxidant activity *(in vitro)* of different parts of *Nelumbo nucifera*. Different results were obtained from the analysis of the total antioxidant activity and reducing activity of lotus parts.

Total Antioxidant Activity

By applying methods reported by Prieto et al. in 1999, the total antioxidant activity of the extracts obtained from different lotus parts was studied using Butylated Hydroxytoluene (BHT) as the positive control, as in Fig. 2. A mixture was prepared using 0.3 ml of each ethanol diluted lotus extracts (ranging from 100-500ug/ml) and a 3 ml solution containing 4 mM Ammonium Molybdate, 28 mM Sodium Phosphate and 0.6 M Sulphuric acid. This mixture was incubated for 90 minutes at a temperature of 95°C, followed by cooling down to 25°C. After that, the absorbance of the resulting mixture was measured at 695 nm.

Reducing Power Assay

This study was conducted with ascorbic acid as the positive control, as shown in **Fig. 3**. About 2.5 ml of deionised water diluted extracts of lotus (ranging from 100-500ug/ml) was mixed gradually with 2.5 ml of phosphate buffer (0.2M at pH 6.6) along with 1% of potassium ferricyanide, followed by incubation for 20 minutes at a temperature of 50°C. After that, 2.5 ml of 10% trichloro acetic acid was added to the solution. Next, centrifugation was performed for 10 minutes at 3000 rpm. Then, 5 ml of the upper layer of the solution was mixed with 0.5 ml of ferric chloride (0.1%) and 2.5 ml of distilled water. Finally, the absorbance of the resulting solution was measured at 700 nm.

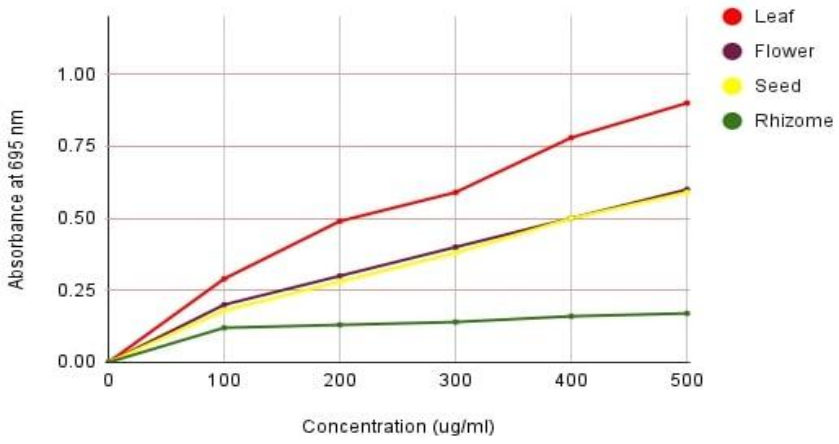

Fig. 2. Antioxidant Activities of different Lotus Parts; Lotus Leaves show the Highest Activity

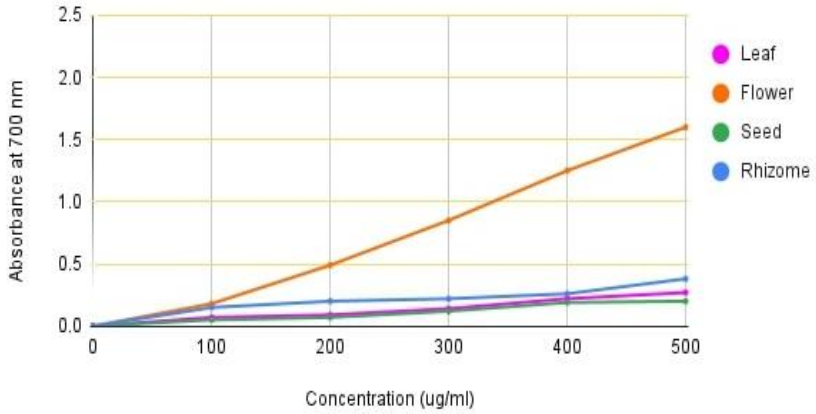

Fig. 3. Reducing Activities of different Lotus Parts, Lotus Flowers show the Highest Activity

THE INTERVENTION OF BIOTECHNOLOGY IN IMPROVING AND ENHANCING THE NUTRITIONAL, ORNAMENTAL AND MEDICINAL AND ECONOMIC VALUE OF LOTUS

Through the application and interference of a number of studies and experimental procedures, including plant breeding, artificial selection, transcriptomic and metabolomic studies, comparative analysis and others, the nutritive, medicinal, ornamental and economic value of lotus can be enhanced. These studies have been discussed in the following paragraphs.

Enlargement of Lotus Rhizomes through the application of Genetics, Total Transcriptome Analysis and Next Generation Sequencing

A study has been conducted for maximizing the yield of lotus and its agricultural production through transcriptomic studies and application of genetics. It mainly focuses on the enlargement of the lotus rhizome. Enlarging the lotus rhizome would lead to the enhancement of the lotus nutritive profile, along with the improvement in its therapeutic index. Not only that, the economic and ornamental value of lotus would be getting improved simultaneously. The phenomenon of rhizome enlargement is almost similar to the tuberization of potato and is maintained through a very complex genetic grid. Through the application of RNA sequencing and transcriptomic studies, gene expressions during lotus rhizome development were studied. This led to the identification of the specific candidate genes required for the enlargement, metabolism, growth and development of lotus rhizomes. From this study, expressions of a total of 22803 genes were observed in different parts of the lotus rhizome. From those expressed genes, indication of other cellular processes, nucleotide binding were indicated through gene ontology. Successful enlargement of lotus rhizome provides important insight into the significant biological pathways and processes in lotus rhizome besides improving the overall economic profile of lotus [50].

Application of RNA Sequencing and Transcriptome Analysis to uncover Alternative Splicing (AS) Events and Single Nucleotide Polymorphisms (SNPs) in lotus

The results of lotus rhizome enlargement simultaneously indicated the roles of alternative splicing and single nucleotide polymorphisms in the development of Asian lotus rhizomes.

Transcriptome sequencing for the identification of Alternative Splicing (AS) Events and Single Nucleotide Polymorphisms (SNPs) was conducted on four Asian Lotus cultivars by the use of Illumina HiSeq-2000 platform. Four Lotus cultivars Bai Ge or BG, Zhou Ou or ZO, Winter Red 1 or WR1 and Red Lingxiao or RL have been considered. In Fig. 4, four such lotus cultivars have been compared. From those four cultivars, about 505 Million pairs of RNA Sequence reads were developed. About 86% of those reads could be mapped to the reference lotus genome. From this study it was revealed that C/T and A/G were the two primary types of SNPs found in the *Nelumbo nucifera* transcriptome. Simultaneously, about 1,77,540 Alternative Splicing Events could be detected in the four *Nelumbo nucifera* cultivars which were dispersed among 64% of the lotus genes expressed. Gene Ontology or GO was conducted to study the genes and their functions which are involved in AS and SNP events. Validation and confirmation of the selected AS and SNP events indicated that about 80% of the AS events and 74% of the SNPs were authentic. This showed the reliability of the RNA Sequencing approach in identifying the gene associated AS and SNP events [51].

RNA Sequencing and Systematic Transcriptomic Analysis to enhance seed development in Lotus and Artificial Selection, Genome-wide Analysis and Comparative Proteomics in the analysis of the petal colouration phenomenon of lotus flowers

In the case of lotus seeds, it is important to increase the production of the seeds and at the same time improving the seed size, since the seeds are an impressive collection of different flavonoids, alkaloids, secondary metabolites and micronutrients, as shown in Fig. 5.

Comparative metabolomics and proteomic studies are being conducted in this aspect, to determine the candidate genes required for enhancing the quantity and quality of lotus seed production. This idea is similar to the idea of enlargement of the lotus rhizome. Deeper studies in this topic will ease the understanding of improving the lotus seed size. Transcriptomic studies have been conducted on two different lotus cultivars, namely Jianxuan-17 and China Antique possessing contrasting phenotypic characters in the number of seeds per seed pod and size of the seeds after particular time spans of pollination. Through this study, about 22,549 genes could be identified, among which there were 2,414 novel genes and 8437 genes were differentially expressed between Jianxuan-17 and China Antique. Gene Ontology (GO) indicated that these differentially expressed genes were important components of Brassinosteroids (BRs) signaling and biosynthesis pathway and could simplify the analysis of gene expression and proliferation. Nine candidate genes involved in the regulation of lotus

seed size were discovered. These genes were responsible for maintaining the size, shape and cell number of lotus seeds [13]. Alongside lotus seeds and rhizomes, studies are also being conducted on lotus flowers.

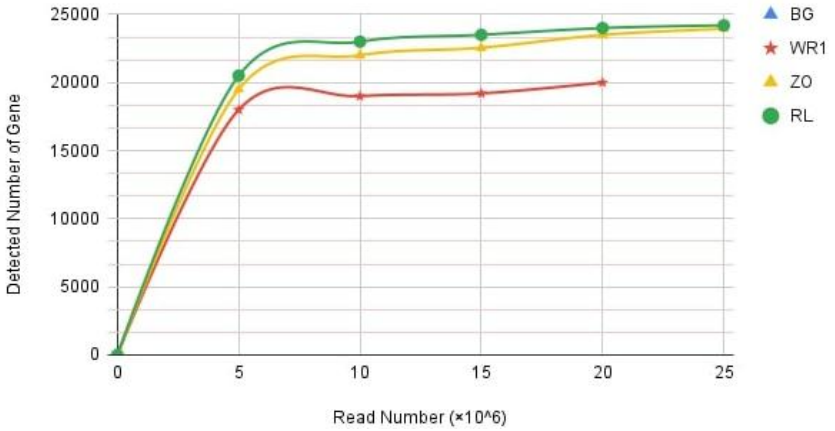

Fig. 4. Graphical representation of correlation between the different read numbers and detected gene numbers

Fig. 5. Variations in the shapes and sizes of lotus seeds

Fig. 6. Variations in the germplasm of lotus flowers displaying different colours and shapes

Fig. 7. Comparison in the number of up-regulated and down-regulated differentially expressed genes between Petal vs Sepal, Stamen vs Petal, Stamen vs Sepal

In the case of lotus flowers, intensive studies are being conducted for analyzing the different pigments and pigment compositions of lotus flower petals, as shown in Fig. 6. With the application of artificial selection, the overall economic value of lotus can be enhanced. From some other studies it was also revealed that the red and yellow colour of the petals are majorly because of the anthocyanin and carotenoid pigments present in the lotus flowers [52]. A very similar anthocyanin

regulatory biosynthesis pathway has been observed in *Arabidopsis* when genome-wide analysis of the *MYB* gene family was taken into consideration [53]. It was further noticed in the same context that an over-expression of *NnMYB5* in *Arabidopsis* can lead to the accumulation of anthocyanin in the flower stalks and immature seeds of lotus [14]. Another comparative proteomic study performed between the red and white lotus flowers revealed that the primary reason for the absence of the anthocyanin biosynthesis pathway in the white lotus flowers is probably due to the expression of the *ANS* gene [54]. Furthermore, it was also observed that methylation levels in the promoter regions of the *ANS* genes greatly differ between the two sets of flowers which gives an indication of epigenetic regulation of the expression of *ANS* gene.

Increasing the overall Economic Value of Lotus through Breeding, RNA Sequencing and Comparative Transcriptomics

Improvement in the flower shape of lotus can enhance its ornamental and economic value. Lotus flowers with various shapes and orientations (few petalled, double petalled, semi double petalled, duplicate petalled, all double petalled) can be obtained through breeding. Among different shapes, the double-petalled and semi double-petalled ones have been found to be the result of stamen petaloid. Comparative transcriptomic studies performed among the stamen, petals and stamen-petaloid of lotus by RNA sequencing lead to the identification of a number of candidate genes that are involved in stamen-petaloid, as shown in Fig. 7. These are the *MADS-box* genes [55].

One *APETALA2* (*AP2*) gene and about 11 *MADS-box* genes were found to be involved with the stamen-petaloid phenomenon. Among all of them, *AGAMOUS, AGL80* and *AGL15* have been found to be positively related to stamen-petaloid; whereas *AGL6* has been reported to be negatively related to stamen-petaloid [55]. Further studies are going on to identify the gene or set of genes that might be responsible for the phenomenon of varying levels of methylation in the *ANS* gene. Different types of lotus display different phenotypic characteristics which eases the study on different traits by providing suitable germplasms required for its breeding. Another genome wide DNA methylation study indicated the probable involvement of epigenetic control on stamen-petaloid though at the same time it could not identify any changes in the *MADS-box* genes methylation levels [10].

Construction of Genetic Maps through Cross-Breeding and development of several Molecular Markers

By crossing between lotuses with different genotypes possessing contrasting phenotype characteristics, a number of genetic maps and grids have been constructed. Through this, an appreciable number of molecular markers *(AFLP: Amplified Fragment Length Polymorphism; ISSR: Inter-simple sequence repeat; SSR: Simple Sequence Repeats; SRAP: Sequence related amplified polymorphism; RAPD: Random Amplified Polymorphic DNA)* having association with the target traits have been developed [56,15,57].

Comparative Transcriptomics to inspect the Regulation and Control of Flowering in Lotus

Comparative Transcript Profiling was being conducted on the development of

lotus buds of two cultivars (WR1 and BG) at two stages (initial stage and fast stage). These analyses were done to control and influence the flowering time in lotus. Uncovering this mechanism of controlling the flowering time in lotus will enhance the overall economic purpose of lotus since earlier or later blooming of lotus will directly influence the agricultural importance of lotus. These studies aim to explore the candidate genes that maintain the flowering time in lotus, which further gives an indication towards a rather complex regulatory genetic network. In this context, a number of photoperiod-related genes including *CCA1, COP1, CO-LIKE, LHY, FT,* the gibberellic acid-related gene *GAI* and the vernalization gene *VIN3* gained importance [12].

Whole Genome Re-sequencing for the Genome-wide detection of SNP and SSR Markers to simplify the understanding of the evolutionary pattern of *Nelumbo nucifera* and boosting the cultivation of the herb

Re-sequencing of 19 specimens from three cultured temperature subgroups of lotus (seed, rhizome, flower), one tropical group of lotus (Thai lotus), one wild temperate subgroup of lotus (wild lotus) and an outer group lotus (*Nelumbo lutea)*. Through polymorphic analysis and study of the lotus genetic diversity, significant results concerning the gene signature, gene evolution, genetic diversity and overall divergence, adaptation, and evolution of different samples of lotus were obtained. Also through Single Sequence Repeats (SSR), Single Nucleotide Polymorphism (SNP) and Insertions and Deletions (InDels), scientists have tried to better the breeding of lotus at a genetic level. In a comparative sequencing between two wild lotus genome samples (Middle Lake Wild Lotus and Chiang Mai Wild Lotus), a total of about 328, 251 InDels, 3,180,059 SNPs and 14,191 structural variants (SVs) were identified. This analysis provides impressive quantities of genomic data, markers and resources for the molecular selection and Quantitative Trait Locus (QTL) identification of the lotus species, which would further enhance lotus breeding and cultivation. Similar studies and experiments on lotus are being conducted continuously to ease the understanding of the lotus herb at a genetic level [58-60].

In Vitro Regeneration of Lotus

Making use of dormant embryos and buds, a high frequency regeneration system of plants was prepared and applied on lotus herb [16]. This regeneration technique was found to be highly efficient and aided in the large-scale production of lotus rapidly and in a much shorter period of time. Its rate of multiplication was noted to be really high. This particular technique was based on the usage of lotus buds for regeneration. Another technique which made use of the dormant lotus embryos helped to ease the issues and problems regarding the cross breeding of lotus. These regeneration techniques used by scientist Yu and his team helped in the conservation of the valuable lotus hybrid seeds for further usage in later times. Another *in vitro* regeneration study was conducted on lotus [61] where the herb could be successfully regenerated from immature explant yellow plumule cultures. Different growth regulators like 6-benzyl aminopurine (BAP) and alpha-naphthalene acetic acid (NAA) were added to the culture media and their effects were studied. New shoots and roots gradually developed after a certain period of time. Effects of double layered mediums and distance of light were simultaneously studied while analyzing the in vitro generation of lotus herb. This study was the very first one to report the

successful culturing of the yellow plumule immature explants on optimum double layered media having solid Murashige and Skoog media (MS media) supplied with 1.5mg/L of NAA and 0.5mg/L of BAP for about 20 weeks, which gradually led to the development of a very new shoot feature (multiple shoots layered) at an optimum light distance of about 200 mm from the 1000 lux influorescent light source which led to the development of the tallest shoot of about (16.67 ± 0.23 mm) along with the highest number of shoots per explants (16.67 ± 0.23 mm).

SECONDARY METABOLITES AND PHYTOCHEMICALS OBTAINED FROM LOTUS

Lotus is a traditionally important perennial herb and is an epitome of abundance of a number of alkaloids and extract contents. Several secondary metabolites including phenolic acids, flavonoids can be extracted from lotus Table 2 making it an important medicinal herb [62,53]. Various system analysis and studies are being conducted to understand and ease the different methods of extraction of these secondary metabolites from different parts of lotus [64-71]. These methods were also useful in studying and analyzing the lotus germplasms of different origins; along with that, the probable uses and importance of the various secondary metabolites of lotus in the medical field, were also analyzed through these methods [72]. Studies have been conducted to ease the understanding of the different biosynthetic pathways in lotus, from which the benzylisoquinoline alkaloids (BIA) biosynthetic pathway gained importance, and it further indicated (through comparative studies) that the transcriptional controls differ abruptly in low BIAs lotus compared to high BIAs lotus [73,74]. On the basis of sequence similarity analysis, a number of genes encrypting the different enzymes involved with the BIA biosynthetic pathway were discovered [75].

Table 2. Following is the list of the various alkaloids, flavonoids and bio-active compounds obtained and extracted from different parts of lotus in Table 2. These extracts can produce different biological effects and are thus very much important in the area of medicine and therapeutics

Part of Lotus	Extracts
Rhizome	Ascorbic acid, riboflavin, thiamine, betulinic acid and niacin.
Seeds	Lotusine, isoliensinine, liensinine, pronuciferine, nuciferine, neferine, dauricine, armepavine, roemerine, procyanidin, saponins, isoquininolinol
Flower	Nelumboroside A and B, glucopyranoside, kaempferol, quercetin, isorhamnetin, myercetin, arbutin, myo-inositol, adenine, B-sitosterol
Leaves	Nuciferine, pronuciferine, N-nornuciferine, norcoclaurine, negferine, nelumboroside, anonaine, roemerine, coclaurine, remerine, liensinines, armepavine, asimilobine, quercetin, leucodelphinidin, leucoanthocyanidin

RESULTS AND DISCUSSION

A good number of studies are being conducted on lotus to explore its usage in different fields- medicine, nutrition, agriculture, ornamental, etc. Different parts of lotus, including its stems, seeds, flowers, leaves, pods etc. are important sources of nutrition, food, therapeutics and medicines. As shown in Table 3, the nutritional value of 100 gm dried lotus seeds have been well evaluated, and in Table 4, the nutritional content of 60 gm boiled lotus rhizome have been analyzed. These informations have been collected from the National Nutrient Database, USDA.

Table 3. Nutrition Profile of 100 gm dried lotus seeds

Contents	Nutrition Value
Energy	332 Kcal
Carbohydrates	64.47 gm
Total Fat	1.97 gm
Protein	15.41 gm
Cholesterol	0 mg
Vitamin A	50 IU
Vitamin C	0 ug
Thiamine	0.640 mg
Riboflavin	0.150 mg
Pyridoxine	0.629 mg
Niacin	1.60 mg
Folates	104 ug
Pantothenic Acid	0.851 mg
Calcium	163 mg
Magnesium	210 mg
Copper	0.350 mg
Manganese	210 mg
Iron	3.53 mg
Zinc	1.05 mg
Phosphorus	626 mg
Sodium	5 mg
Potassium	1368 mg

Table 4. Nutrition Value of 60 gm boiled lotus rhizome

Contents	Nutrition Value
Calories	40 Cal
Carbohydrate	9.6 gm
Protein	1 gm
Fat	0 gm
Vitamin B	0.2 mg
Vitamin C	16.4 mg
Sugars	0.3 gm
Sodium	27 mg
Fiber	1.9 gm

These lotus parts and their extracts can produce different effects after consumption and usage: anti-inflammatory, arrhythmic, anti-depressant, anti-fibrosis, anti-proliferative, anti-viral, anti-obesity, anti-oxidative and other various effects. Nelumboroside A and B, glucopyranoside, lotusine, kaempferol, quercetin, nuciferine, pronuceferie, isorhamnetin, armepavine and other many alkaloids, flavonoids and phenolic coupons are extracted and isolated from lotus. These extracts can not only treat a number of complications and diseases, but also with time that are proving to be useful in controlling other complexities in the body. Importance of different parts of lotus, lotus extracts has been well described in this study. Along with that, the cross connection of biotechnology with lotus agriculture has been depicted through the description of the various studies and analysis that are being conducted on lotus.

CONCLUSION

Though various advancements have been made in studying the lotus herb through different biotechnological and molecular strategies, unfortunately enough, there are still some drawbacks which need to be updated. The molecular biology analysis of lotus still lacks valid information to carry out more specified experimental works. But through continuous advancement in this field, it can be believed that successful experimentation on lotus will actually enhance the quality of lotus production in agriculture science along with the enhancement of the nutrition profile of lotus in food science and therapeutic index of lotus in the field of medicine. With the ongoing research and studies, it can be well assumed that with time, the importance and uniqueness of lotus is going to gain more popularity in the area of biological sciences so that further studies can be carried out in the areas which are still left unexplored.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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