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# A Review on *Allium cepa* and Biological transformation of its lachrymatory Effect

**Ghalia Nawal*, Kausar Malik, Hira Naeem**

Department of Biotechnology, Lahore College for Women University, Pakistan
*Corresponding author: *ghalianawal@yahoo.com*

## Abstract

Onions are second most important cash crop of monocotyledonous and perennial plants, eminent for their pungent flavor and lachrymatory effect ,due to presence of volatile group of amino acids Alkenyl cysteine sulphaoxides (ACSOs) which are cleaved by an enzyme allinase upon damage to cells. To evade this lachrymatory effect through biological transformation involves silencing the Lachrymatory factor synthase (LFS) gene present on chromosome 5 on two different loci mainly by *Agrobacterium* mediated manipulation and biolistic gene delivery technique.Transformants (tearless ,sweet onions with low lachrymatory factor and sulfur content) are analyzed using Polymerase chain reaction (PCR) and Fluorescence *in situ* hybridization (FISH) while percentage of Lachrymatory factor (LF) is analyzed with help of Gas chromatography. The transformed cells show normal pattern of mendelian inheritance. Although these genetically modified (GM) onions have shown a 15% increased demand but they have reduced antiplateletic, antimicrobial,antifungal and wound healing properties along with pungency and lachrymatory effect.

**Keywords:** Alkenyl cysteine sulphaoxides (ACSOs), Allinase , Lachrymatory factor synthase (LFS), Biological Transformation methods, GM Tearless onions.

## Introduction

Consumption of Allium species in particular (onions and garlic) by the prehistoric civilizations is evident through their narration in Quran and other holy books. The word Allium is derived from Greek word, which means “to avoid” because of their Pungent smell and tearing “lachrymatory effect” upon their cutting for usage. The members of Allium family are perennial, monocots and consists of nearly 600-750 species, making it one of the largest plant genera. The main Allium species are Onion and garlic which are famous for their Lachrymatory effect and requisite flavor in cooking. Onions are major food components of many food cuisines and starting material for Punjabi cuisine. Maximum of its biological diversity is observed in regions of Afghanistan, Iran, India, Pakistan and china with changes in its size, shape and color.

### Global Production:

On global scale the entire production of two major allium crops i.e. Onion and garlic was nearly 85 million in 2007 accounting 66 million for the former and 16 million for the later one within 175 countries. Due to ease of their storage aiding export, Onions are always traded more this quality makes them second most significant cash crop after tomatoes. But storing them for longer than half a year is troublesome with post harvest spoilage estimation of about a quarter i.e. 25-30 percent of the yield.(Griffiths et al., 2002).

### Phytochemistry:

The Biochemical nature of onions reveals that they have no fat but carbohydrates (5.2-9%), Ash (0.6%) and 30 calories per serving. Various mineral elements found within 100 mg of fresh weight are Calcium 190-540, Phosphorus 200-430,Potassium 80-110,Sodium 31-50,Magnissum 81-150,Aluminium 0.5-1,Barium 0.1-1,Iron 1.8-2.6, Strontium 0.8-7, Boron 0.6-1,copper 0.05-0.6,zinc 1.5-2.8,magnanesse 0.5-1.0 and

Sulfur 50-51 mg. while some of the vitamins found per hundred gram are Vitamin D 0.3,Riboflavin 0.05,Nicotinic acid 0.2, Vitamin C 10.0, Folic acid 16.0, Biotin 0.9 and Pentothenic acid 0.14 milligrams along with moisture content of 88.6-92.8 %.

## Lachrymatory Effect:

Out of many amino acids found in onion like lysine and glysine the arginine and glumtamic acids are more in concentration especially in central area of bulbs.The distinguishing flavor along with specific pungency is due to non protein sulphur and its related compounds. Everyone who have tried cutting or chopping onions has experienced the lachrymatory "tear stimulating effect of onions which is only produced on disruption of cells. This Effect is never generated by undamaged cells.

Onions have pungent smell and specific aroma due to richness of specific odourless, non-volatile amino acids of general name as Alkenyl cysteine Sulphoxides (ACOS) and Flavinoids.The Flavinoids are the anti-oxidants that help to delay oxidative damage to the cells by eliminating free radicals and they are further categorized into anthocyanins that results in purplish or red colour in some varieties while the other group contains quercetin and its derivatives which are responsible for the yellow and brown skins of many other varieties. Mainly the Alkenyl cysteine Sulphoxides (ACOS) are the flavour precursors and also responsible for generating the lachrymatory effect (the tear stimulating properties) produced on cutting of Onions and garlic.The Alkenyl cysteine Sulphoxides compounds are generally present in four forms confined to cytoplasm, which are cleaved by the enzyme allinase confined to cell vacuole, that produce the specific odour and aroma of onion when disruption of cells causes contact of flavor precursors (ACOSs) with the enzyme. The end products of this reaction is a mixture of certain chemical compounds including thiosulphinates, thiosulphonates, mono-sulphides, di -sulphides and tri-sulphides. These compounds are produced upon rupturing of tissues as a defense by providing protection against various kinds of fungus and arthropods species. Along with bulb these Alkenyl cysteine Sulphoxides (ACOS) responsible for pungency are actually synthesized in leaf blades and transported to bulb, roots and base plate of onion while their complete absence is observed in seeds.The level of these Sulphaoxides is maximum in leaf blades especially during bulbing and eventually starts dropping during maturity and sprouting (Brewster 1994).

A variety of environmental factors most notably photoperiod, temperature, fertility status, Humidity and amount of sulfur contents in soil effect flavor and quality of onions. A higher temperature, optimum photoperiod and low moisture content of soil results in onions of greater pungent flavor. That is why the onions of Pakistan and India have superior pungency and eminence (Freeman and Whenham, 1976).

Chiefly onions are used for cooking purposes in almost all regions of the world, while the ancient civilization also used them for treating wounds and several stomach related disorders. The integrated Compounds analyzed in onion have been reported to have a range of health advantages that include anti-carcinogenic properties, anti-platelet activity, anti-thrombotic activity, anti-asthmatic and antibiotic effects.These anti-thrombiotic and anti plateletic effects aids in avoidance and treatment of Heart diseases by preventing atherosclerosis.Various scientific and pharmacological researches on the onion biochemistry have suggested presence of antimicrobial and anti-fungal properties. Although onions have beneficial impact on human health but they are harmful for some domestic animals like for Dogs, Cats, Sheeps, calttles and Goats due to their toxicity. If large quaintly of is consumed by these animals it can impair the oxgen transport system within blood and may result in anemia as well (Brewster 1994).

## Biological Transformation Techniques:

The term genetic transformation refers to cover the systems that transfer a specific set of characterized genes through Agrobacterium-mediated or biolistic gene delivery techniques to manipulate the genetic makeup of a particular specie. Over the past years many huge number of techniques and protocols are available for transforming model plant species and creating genetically modified plants (Horsch et al., 1985). But those protocols and techniques are not applicable to manipulate the genetic makeup of all crop species .Earlier protocols for *Agrobacterium tumefaciens*-mediated transformation did not work on monocotyledonous plants until they were modified (Hiei et al., 1997).Resulting in inventing many alternative techniques to transfer DNA to plant.

There are basically two methods of DNA transfer: direct DNA delivery and vector-mediated DNA delivery. Direct DNA delivery uses physical, chemical or electrical methods to deliver DNA directly into the plant cell (Songstad et al., 1995). Once in the cell,

only intracellular processes are available to facilitate DNA integration into the host genome. Of the many direct DNA delivery techniques available, the most commonly used is biolistic gene transfer, where a gene gun is used to shoot tiny DNA-laden gold bullets into the plant cell.. By 1990, stable transformation of maize and soybean had been reported using this technique (McCabe et al., 1988; Fromm et al., 1990). Different types of gene guns have been developed (Vain et al., 1993), but the PDS1000 helium biolistic gun (Dupont) is the most widely used. Since the 1990s, biolistic gene transfer has gained favour, particularly for the transformation of monocotyledonous crop species (Christou, 1995). But results produced by this that have been difficult to repeat and produces transformants that contain large numbers of unwanted integration events, such as the insertion of multiple and/or faulty copies of the transgene into the host genome, which prevent the recovery of phenotypically normal plants (Spencer et al., 1992).

Vector-mediated DNA delivery involve the use the natural ability of certain microorganisms to mediate the successful transfer and integration of foreign DNA into the host plant. By far the most frequently used of the vector-mediated techniques is Agrobacterium-mediated transformation. Agrobacterium strains, containing a tumour inducing (Ti) plasmid, have the ability to transfer a specific region of that plasmid, the T-DNA, to plant genomes. Under natural conditions, the Ti plasmid contains virulence genes that, with the help of chromosomal-based bacterial genes, effect the transfer process. The T-DNA sequences transferred contain flanking DNA sequences that assist in the integration process and genes that enable the affected plant cell to proliferate and produce a carbon source for the Agrobacteria. By manipulating this process it has been possible to substitute the wild type T-DNA region with modified T-DNA containing genes or sequences of choice (Christou, 1995). Using particular strains of Agrobacterium in combination with specific virulence genes and susceptible host-cell tissue types, it has been possible to broaden the host range of the Agrobacterium-mediated gene-transfer process (Hooykaas et al., 1984). In 1994, the first routine transformation system for monocotyledonous plants was developed (Hiei et al., 1997) and this has led to the resurgence in popularity of this technique. Nearly all transgenic crop species have been produced using versions of the biolistic or Agrobacterium-mediated transformation systems. But these methods are costly, difficult and endearing.

In addition to these techniques scientists are focusing to use the natural ability of transposon sequences to 'jump' genes from extrachromosomal plasmid DNA and integrate into plant genomes (Lebel et al., 1995).

Other methods of targeted integrations and site directed recombinations are also being developed (Puchta, 1998). At present, only Agrobacterium and biolistic methods of transforming alliums have been implemented.

## Transformation of Onion pungency:

Upon cutting of onion *Allium cepa* with knife results in lachrymatory smell stimulating tearing. Factor responsible for tearing was identified more than 40 years ago as propanthial-*S*-oxide, is produced during the enzymatic conversion of 1-propenyl sulfenic acid, a putative reaction product of alliinase acting on *trans*-1-propenyl cysteine sulfoxide (*trans*-PRENCSO) through lachrymatory factor synthase (LFS).This Factor causes cleavage of ACSOs by enzyme alliinase, upon disruption of the cell, produces volatile flavours, odours and lachrymatory compounds ('pungency'), as well as pyruvate and ammonia (Clark et al., 1998). Some of the first compounds produced upon lysis of the cell are the thiosulphinates, which subsequently produce the cascade of additional organosulphur products that make up some of the above compounds. With beginning of advances in scientific research efforts for tearless onions or producing low-LF onion were making their way. Suppression of LF production would cause an increase in thiosulfinates and sweetness.

Understanding of onion's biochemistry along with a knowhow of the in charge genes allow researchers to transform pungency of onions. Onions contain 16 chromosomes and its genome is prominent for its great size ,which is 15 Gega base pairs per Chromosome .Due to huge size of its genome its Genomic studies of onions are tricky as onions have huge size og genome ,out-crossing and very heterozygous. PCR-based map has exposed the LFS gene on chromosome number 5. The physical distribution of AFLP markers along Allium chromosomes has been studied via the integration of recombination and physical maps in a trihybrid population, *A. cepa* × (*A. roylei* × *A. fistulosum*) (Khrustaleva *et al.* 2005). Direct physical mapping of genes on onion chromosomes is restricted due to the genome abundance with repetitive elements (Stack and Comings 1979;Pearce *et al.* 1996). For the detection of specific loci Fluorescence *in situ* hybridization (FISH) was successfully applied using large genomic clones as probes mostly in plant

species with small gene-rich genomes, such as *Arabidopsis thaliana* (Kornneef *et al*. 2003) or rice (Jiang *et al*. 1995).Furthermore, a BAC-FISH study using two BAC clones bearing LFS genes as a probe showed that LFS genes are localized in the proximal region of the long arm of the chromosome. Scientific research using multiple approaches to map LFS genes has revealed that LFS in onion is transcribed from at least two loci and that they are localized on chromosome 5.

Current silencing of LSF is done by regenerating transformed plants containing antisense versions of the alliinase gene in order to see whether this type of manipulation can result in gene silencing and be used to modify onion pungency. Other enzymes, such as gamma glutamyl cysteine synthetase, glutathione S-transferase and -glutamyl transpeptidase, involved in the production of the ACSOs, are also being investigated for alteration of their pathways (Lancaster and Shaw, 1994). Other plant species, including the brassicas, are known to produce ACSOs (Maw, 1982) and are thought to have a similar sulphur pathway leading to the production of methyl cysteine sulphoxide.

Colour, size, shape, number, thickness and adhesion of skins, storage abilities, solids content, quercetin levels, pungency and sweetness are all traits that breeders would like to manipulate. The very existence of white, yellow and red onions indicates that the anthocyanin-based colour pathway is present and so it should be possible to introduce proven anthocyanin regulatory genes to specifically modify Allium colour, as has successfully been done in other plants (Tanaka et al., 1998).

## Gene Delivery:

Both vector-mediated and direct gene-transfer systems have been applied to alliums with some success (Eady, 2001). However, to date, only the vector-mediated Agrobacterium system has been reported to be repeatable and to work on more than one cultivar. (Myers and Simon, 1998) used the PDS 1000 helium particle gun (Dupont) as a direct gene-transfer system to produce a transgenic garlic plant. However, as with similar work in onions, this system is very inefficient and requires the transformation of a specific cell line. In the case of garlic, regeneration takes about 13 months, which increases the chance of producing undesirable soma-clonal variation. There have also been claims that transgenic leek plants have been produced using particle bombardment.

Recently, in Arabidopsis, an in vivo technique has been developed whereby the floral tissues are simply dipped in a modified Agrobacterium solution and then allowed to develop. Up to 3% of the seed produced can be transgenic (Clough and Bent, 1998). In other developments, researchers are using transposon sequences to ‘jump’ genes into the desired genome (Houba-Herin et al., 1994) or they may use homologous recombination systems to direct site-specific gene integration (Hooykaas, et al 1984). Ultimately one of these systems may prove to be more effective than using *Agrobacterium.*

## Gene Regulation:

Numerous regulatory sequences are now available to direct foreign gene expression in plant cells. Many of these have been isolated and modified into a ‘cassette’ format so that the gene to be expressed can simply be slotted downstream of the promoter of choice, e.g. the pBIN series of binary vectors and the pCambia series. It is important to use sequences that produce a high level of selective gene product at a later stage when the transformed material is being selected. The ability of available plant regulatory sequences (promoters, introns, leader sequences) to direct gene expression in onion cells has not been studied in detail, although some information on commonly used promoter sequences has been obtained from bombardment studies in onion and garlic (Eady et al., 2001). These reports concluded that the cauliflower mosaic virus (CaMV) 35S promotional sequence drives high levels of expression in Allium tissue. This sequence and the nos promotional sequence have both subsequently been used in successful Allium transformation studies (Eady et al., 2001). Fusing gene promoters, enhancers and other regulatory sequences (either from Allium genes or from other origins) to reporter genes, such as the gfp gene (Haseloff et al., 1997), and studying the expression of such introduced constructs,makes it possible to induce precise spatial and temporal specific transgene expression patterns in alliums.

## Culture System:

In vivo culture of onions involves clonal propagation from multicellular meristems. It is preferable to obtain transgenic plants by integrating DNA into a single totipotent cell and then regenerating a complete plant from that cell. The cell has to be competent both for accepting DNA and for regeneration. The alternative to this is when the cell is competent to accept DNA but can regenerate only as part of an existing multicellular structure. In this case, a chimeric tissue is

produced as the primary transgenic material and independence to regenerate proceeds when the transgenic cell mass reaches a particular size or developmental stage. In reality, totipotency can only be truly observed in isolated protoplasts. In other systems, it is difficult to determine the precise role of adjacent cells, although it is obvious that some systems are more dependent on surrounding cells than others. Callus (or dedifferentiated) cells provide useful sources of independent cells. However, regeneration from such starting material to a phenotypically normal plant can be difficult. The major Agrobacterium based monocotyledonous transformation protocol claims to use embryo-derived callus material, which, by definition, is a dedifferentiated uniform cell line. Such a system is unlikely to work with Allium. Mature or immature embryo or embryo derived cultures as a source of dual transformation/regeneration-competent cell types. These types of cultures have recently been reported for several Allium species. One problem associated with this difficult culture process is that of somaclonal variation, which may arise from the long and complex culture regime.

## Selection of transgenic tissue:

Transgenic plants are usually selected by using either antibiotic or herbicide resistant gene constructs. Initial investigations indicate that herbicides such as geneticin, hygromycin or phosphinothricin could all be useful selective agents for transgenic Allium selection (Eady and Lister, 1998). Since then, the nptII gene has been successfully used to confer resistance to the antibiotics paromycin (Myers and Simon, 1998a) or geneticin (Eady et al., 2001). The bar gene has also been used to confer resistance to the herbicide phosphinothricin. Groups who are concerned about the use of antibiotic resistance to develop commercial crops favour the use of herbicide resistance as the selectable marker. This too has its limitations, especially if it becomes desirable to ‘pyramid’ genes (i.e. to insert additional genes into already transformed plants). Other selection systems have recently been developed in plants, including the use of specific nutritional requirements in the regeneration media, e.g. the phosphomannose isomerase (PMI) gene as the selectable gene and mannose as the selective agent (Joersbo et al., 1998) and visual reporter genes (Vain et al., 1993). These have not yet been tested on alliums. In addition, removable selection systems are being developed, e.g. by cotransformation, site-specific recombination and transposon-mediated systems. The selective gene can be removed at a later stage, leaving only the gene of choice. This process allows multiple alterations to be made to a particular cultivar. The speed with which these developments can be applied to alliums remains to be seen.

## Exflasking:

Transferring the primary transformant from in vitro culture to the glasshouse is often a technically difficult process. Fortunately, Allium plantlets in culture are quite robust and there are numerous reports of successful transfer to the glasshouse (Novak, 1990). Two techniques are used in our laboratory. They are based on either the transfer of vigorously growing plantlets or of in vitro bulbs produced by culturing the plantlets on Murishige and Skoog medium (MS) plus 120 g l−1 of sucrose (Seabrook, 1994). For these processes to be successful, it is essential that the glasshouse is warm (12–23°C day, 4–16°C night) and has at least 12 h of bright daylight.

## Agarobacterium mediated Transformation:

*Agrobacterium tumefaciens* strain LBA4404 containing the plasmid binary vector pBIN or pCambia derivatives have been used in Allium transformation experiments. Overnight, Agrobacterium cultures grown in Luna broth (LB) media (Sambrook et al., 1989) containing appropriate selective agents (e.g. Eady et al., 2000) were replenished with an equal volume of LB containing antibiotic and 100 M acetosyringone (virulence-gene-inducing factor) and grown until they reached an optical density of about 1.0 at 550 nm. Agrobacteria were isolated by centrifugation and resuspended in an equal volume of liquid embryogenic induction medium (P5) (Eady et al., 1996) containing 200 M acetosyringone. Immature embryos from field-grown umbels of bulb onion cv. ‘Canterbury Longkeeper’ were isolated under a stereomicroscope . The embryos used were from immature seeds at the stage of recently blackened seed-coat. and with the endosperm still liquid. They were removed from the ovaries, cut into ~1 mm lengths and transferred in batches of 40 into 0.8 ml of Agrobacterium solution, vortexed for 30 s and placed under vacuum (~25 mmHg) for 30 min. These tissue pieces were then blotted dry on filter-paper before transfer to P5 media. After 6 days of cocultivation with the bacteria at 28°C in the dark, embryo pieces were transferred to P5 containing appropriate selection agents in order to select for transgenic tissue and eliminate Agrobacteria. Embryo pieces were cultured in the dark under the same conditions described for the production of secondary embryos (Eady et al., 1998b), with transfer to fresh

medium every fortnight. After ~8–16 weeks, actively growing material (also identified using visual-marker gene expression, if appropriate) was transferred to regeneration medium containing the selective agent 20 mg l−1 of geneticin when using the nptII gene. Shoot cultures were maintained for 12 weeks, and developing shoots were transferred to MS media (Murashige and Skoog, 1962) plus selective agent to induce rooting of transgenic shoots only. Rooted plants were either transferred to MS plus 120 g l−1 sucrose to induce bulbing or to soil in the glasshouse (12 h 12–23°C day, 12 h 4–16°C night). In vitro bulbs could be maintained for many months on the media and transferred to the glasshouse when appropriate.
Increasing day length induced bulbing in glasshouse-grown plants naturally. After 50% of the tops had fallen, bulbs were lifted and air-dried. Bulbs greater than 45 mm in diameter were cold-stored at 4°C for 3 months to induce floral meristems prior to planting. Plants from all transformants, produced using the above technique, have grown in a phenotypically normal fashion and produced scapes and umbels. Flowers were self-pollinated by enclosing individual umbels within microperforated plastic bread bags containing green bottle flies. Seed was collected 2–3 months later from dried umbels.

## Detection of transgene:

Initially, the presence of the transgene in putative transgenic onion tissue was screened using the polymerase chain reaction (PCR) in order to amplify specific fragments of a particular DNA sequence. However, at present, it is still routine to determine transgenic status conclusively by Southern blot analysis. After about 2 months' growth in the glasshouse, transgenic leaf material (approximately 1 g) was collected and Southern blot analysis was performed on putative transgenic plants. Plants have been screened for the presence of introduced nptII, gfp and bar genes. In all cases, integrations have been observed in copy numbers (number of integrations per genome) similar to those observed in the transformation of other plant species.

## Stability of transgene:

Transformants produced in initial experiments have grown to maturity and appear phenotypically normal.self fertilization of these independent transformants plants, as F1 seed has recently been collected and germinated. Initial results indicate that the transgene is usually inherited in a normal Mendelian fashion.

## Impact of GM onions:

With respect to the production of food crops concern exists among the public about the risks of genetic engineering, with use of microorganism's ability to create genetically modified organisms to produce custom-made products, without any negative response from the community. On the other hand, genetic engineering does have the potential to help to feed the 3 billion people who will be born in the next three decades (Kendall et al., 1997). Deliberate introductions of highly specialized plants and animals have been made around the world, in fact the global agricultural system as we know it today has depended upon this (Diamond, 1998). The introduction of crop species has caused relatively little concern, as they are generally not adapted for existence outside the field environment. Without such introductions, our lifestyles would be very different. In contrast, the introduction of highly evolved wild plant and animal species into unmanaged ecosystems has caused severe modifications to the native flora and fauna. The manipulation of crop species, to alter only a few well-defined characteristics, is highly unlikely to convert them into organisms as invasive as the highly evolved wild species and therefore would not significantly improve their chances of surviving in a natural habitat. There are fears that GM foods may be toxic, despite the requirement for rigorous testing regimes that are more comprehensive than any previously implemented for other crops. Yet, in the evolutionary struggle, life forms have become masters of biochemical warfare and even innocuous crops can contain toxic surprises which need precise processing to ensure their elimination, e.g. in kidney beans, cassava and potatoes.

The 'developed' world is supported primarily by produce from intensive agriculture. This agroindustry relies heavily on the use of fossil fuels and the application of large quantities of toxic chemicals, each with its own risks. This scenario is unlikely to change rapidly, even with the adoption of more environmentally friendly methods of agricultural production. So the risks of developing or not developing GM crops should be compared with those presented by our current, less than perfect systems and with other emerging alternatives. What follows is a brief discussion of some of the key concerns expressed about GM crops as they relate to onione potential for GM onion crops to become weeds Onions possess very few, if any, weedy characteristics, such as seed dormancy, broad adaptation, indeterminate growth, continuous flowering, seed production and dispersal.

The occasional volunteers from leftover bulbs, which grow following onion production, rarely survive to produce seed. Seed viability declines quickly in open storage and onion seedlings will not thrive because they are not competitive with other plants. As it is not intended to introduce genes conferring weedy characteristics into onions, it is highly improbable that onions, GM or otherwise, will ever become a major weed problem. In the USA, only A. vineale from the Allium genus is considered a weed that is difficult to control and it multiplies by topsets rather than by seeds.

## Conclusion

Modern Genetic engineering methods have enabled scientists to transform the pungency of Onions *Allium cepa* through a variety of techniques resulting in production of tearless or sweet onions which reveal low concentration of organosulfur compound and Lachrymatory synthase factor. Global demand for these transformed sweet onions has been increased to 15% in recent years due to ease of their use .while on other hand these onions does not possess their natural specific flavor and aroma, have slight mellowness and are rich in thiosulfates that provoke health benefits thought their antithrombic, antiasthematic, antimicrobial,antifungal and wound healing properties.

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