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Transgenics in Phytochemical Defence.

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ABSTRACT

Biotic stress is one of the most significant causes leading to yield loss in plants. Various defence mechanisms operate in plants which can help tolerate or resist stresses. Secondary metabolites secreted by plants play a major role in counteracting pests and pathogens. Scientists around the world have defined strategies to inculcate mechanisms available in resistant plants into the susceptible ones. Genetic engineering of plants has become the recent trend in research to improve the defence mechanism in plants. The paper is an attempt to review on works reported in the development of transgenics towards improvement of plant defence.

Keywords: Terpenes, Phenolics, N-containing compounds, Transgenics

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INTRODUCTION

Ecosystem is a complex set of relationships which exists among the living organisms. During the course of evolution, various interactions have emerged to maintain the balance in the ecosystem. Some interactions prove to be advantageous to the organisms whereas some are not. In order to cope up with the unfavourable interactions, plants have evolved various defence mechanisms.

Plants counteract various biotic or abiotic stresses, either directly or indirectly. Direct defences basically include specialized appendages (mechanical defence) and the release of toxins whereas indirect defences include release of volatile chemicals and 'extra floral nectar' to attract the natural enemies of the herbivores, thereby protecting the plant [1]. Both the types of defence mechanisms may be constitutively present or transiently wound-induced. Current research is mostly focused on the indirect defence mechanisms.
Plants have evolved various morphological traits [2] such as:

- Thorns (Honey Locust Tree)
- Spines (Cactus)
- Prickles (Rose)
- Wax (Bull Bay)
- Trichomes (Dusty Miller, Soyabean)

Certain chemicals present in the plant are not involved in growth and reproduction but their absence may result in long-term impairment. These are termed as secondary metabolites which are involved in chemical plant defence mechanism by acting as toxins which affect the physiology of the herbivores. The secondary metabolites can be constitutively stored (phytoanticipins) or insect/microbe-induced (phytoalexins) [1].

Most of the secondary metabolites which are involved in plant defence mechanism are categorized into three major categories:

- Terpenes
- Phenolics
- N-containing compounds

TERPENES

The first major category and by far the largest group of secondary metabolites [2, 3] involved in plant defence mechanism comprises of terpenes which are naturally occurring hydrocarbons [4]. The isoprene unit forms the backbone of terpenes which are volatile gases [2]. Mainly biosynthesized by the 2-c-methyl-1-D-erythritol-4-phosphate (MEP) pathway [3], terpenes (monoterpenes and sesquiterpenes) are the primary components of the essential oils. They contribute to fragrance of plants, act as insect toxins and protect the plants from herbivores [2]. They are classified according to the number of carbon atoms or the number of C_5 isoprene units (multiples of C_5H_8) as shown in Table 1. [4, 5].

Table 1. Classification of Terpenes

Sl. No.	Class	Examples
1	Hemiterpene/Isoprene • One isoprene unit	Oxygen containing derivatives – Prenol, Isovaleric acid
2	Monoterpenes • Two isoprene units • $C_{10}H_{16}$	Menthol, Geraniol, Limonene, Terpineol
3	Sesquiterpenes • Three isoprene units • $C_{15}H_{24}$	Farnesene, Farnesol
4	Diterpenes • Four isoprene units • $C_{20}H_{32}$ • Derived from Geranyl Geranyl Diphosphate • Have anti-microbial and anti-inflammatory activity	Gibberellic acid, Cafestol, Kahweol, Cembrene, Texadiene
5	Sesterterpenes • Five isoprene units • $C_{25}H_{40}$	Geranyl-farnesol, Abscisic acid
6	Triterpenes • Six isoprene units • $C_{30}H_{48}$	Squalene, Sitosterol, Yamogenin
7	Tetraterpenes • Eight isoprene units • $C_{40}H_{64}$	Carotene, Lycopene
8	Polyterpenes • Long chains of multiple isoprene units	Natural rubber

Some examples [3,4]

- Pyrethroid - a monoterpene ester found in the leaves and flowers of *Chrysanthemum* species
- Limonene - a monoterpene constituent of lemon oil
- Peppermint and spearmint – Monoterpenes found in *Mentha spp.*
- Basil – Monoterpene found in *Ocimum spp.*
- Oregano - Monoterpene found in *Origanum spp.*
- Gossypol - Diterpenoid produced by cotton (*Gossypium hirsutum*)

PHENOLICS

The second category of secondary metabolites which are a part of the plant defence mechanism include phenolics which are aromatic benzene ring compounds with one or more hydroxyl groups produced by plants mainly for protection against stress. There are various other functions played by phenolics which include lignin and pigment biosynthesis, providing structural integrity and scaffolding support to plants. Importantly, phenolic phytoalexins, repel or kill many microorganisms [6]. Defence related pathways such as the shikimic acid and malonic acid pathways, produce compounds like flavonoids, anthocyanins, phytoalexins, tannins, lignin, and furanocoumarins which are grouped together as phenolics. The classification of phenolics is as mentioned in Table 2.

Table 2. Classification of Phenolic Compounds

Sl.No.	Phenolic compounds	Mechanism of action	Examples
1	Flavonoids	Limits assimilation of dietary proteins and inhibits digestive enzymes of insects [7].	Rotenone (Rat poison) Medicarpin , Rishitin , Camalexin [1, 2]
	(a)Anthocyanins		Pelargonidin, Cyanidin, Peonidin, Malvidin, Delphinidin [5]
	(b)Isoflavonoids		Isoflavones in legumes act as phytoalexins which can be produced in *Arabidopsis*, tobacco and maize. These lack the ability to synthesize these compounds, by overexpression of isoflavone synthase, a cytochrome P450 enzyme [3, 8, 9].
2	Tannins	Bind to salivary proteins and digestive enzymes including trypsin and chymo-trypsin resulting in protein inactivation [2]. Tannins are especially prone to oxidize in insects with high pH guts, forming semi-quinone radicals and quinones, as well as other reactive oxygen species (high levels are produced, leading to toxicity) [10].	Ellagitannins, Gallotannins (classes) [10]
3	Lignin	Insoluble, rigid, and virtually indigestible in pathogen digestive tract [2].	Psoralen [2]
4	Furanocoumarins (activated by U.V radiation)	Highly toxic to herbivores due to integration of these compounds into DNA leading to rapid cell death [2].	Xanthotoxin, Sphondin, Angelicin

NITROGEN-CONTAINING COMPOUNDS

N-containing compounds are characterized by the presence of "Nitrogen" in their structure which is basically derived from amino acids such as aspartate, lysine, tyrosine, and tryptophan [2]. They are classified into two main groups namely alkaloids and cyanogenic glycosides.

Alkaloids

A few important classes of alkaloids found in plants are grouped as:

1) Terpenoid Indole Alkaloids: They have an indole moiety provided by tryptamine (derived from tryptophan) and a terpenoid component.
   Examples: Ajmaline (*Rauwolfia serpentina*), quinine (*Cinchona officinalis*) [11]
2) Benzylisoquinoline Alkaloids: It is the structural backbone of many alkaloids. Plants producing these alkaloids make use of two units of L-tyrosine in their biosynthetic pathway.
   Examples: Morphine and codeine (*Papaver somniferum*), berberine (*Berberis vulgaris*) [11]
3) Tropane Alkaloids: These alkaloids are derived from tropane which is a nitrogenous bicyclic organic compound. They usually occur in plants belonging to the families Erythroxylaceae and Solanaceae [11].
   Examples: Cocaine, Scopolamine [12]
4) Purine alkaloids: The purine alkaloids have nitrogen containing ring structure resembling that of purine. They are termed as pseudo-alkaloids since they are not derived from amino acids like true alkaloids [11].
   Examples: Caffeine, Nicotine [11]
5) Pyrrolizidine alkaloids: Primarily restricted to Boraginaceae, Asteraceae, Fabaceae, and Orchidaceae. Its mostly derived either from the polyamines putrescine and spermidine. These are strong deterrents to insects and vertebrates. They may exist in two molecular forms, the protoxic free base and the non-toxic N oxide. Most plants produce and store pyrrolizidine alkaloids as N oxides. These are easily reduced in the gut of a herbivore and absorbed by diffusion as a lipophilic free base into the blood and the haemolymph [13].
   Example: Jacobine (*Senecio jacobae*)
6) Other alkaloids: Quinolizine, steroidal glycoalkaloids [11]

A few examples of alkaloids are mentioned in Table 3.

Cyanogenic glucosides

Cyanogenic glycosides gets hydrolyzed and results in release of hydrogen cyanide, glucosidases and hydroxyl-nitrile lyases. Hydrogen cyanide is a lethal chemical that halts cellular respiration in aerobic organisms whereas glucosidases and hydroxyl-nitril lyases are stored in the edible tissues of plants [2].

Table 3. Examples of alkaloids [2]

Sl.No	Alkaloids	Effects	Examples
1	Caffeine	Toxic to both insects and fungi. Causes allelopathy in coffee seedlings.	*Coffea arabica* (Coffee) *Camellia sinensis* (Tea) *Theobroma cacao* (Cocoa)
2	Nicotine	Released when herbivores graze on the leaves and break open the vacuoles.	*Nicotiana tabacum* (Tobacco)
3	Atropine	Neurotoxin and cardiac stimulant.	*Atropa belladonna* (Deadly Nightshade)
4	Capsaicin	Produces characteristic burning sensation.	*Capsicum* (Chilli peppers)

Table 4. Attempts on creating trangenics for improvement of plant defense [14]

Sl. No.	Host	Source	Transgene – Function	Technique Involved	Effect	Reference
TERPENIODS						
1	*Nicotiana tabacum*	*Fusarium sporotrichioides*	Trichodiene synthase gene Catalyses the conversion of farnesyl pyrophosphate to trichodiene	Over expression - Inserted under constitutive action of CaMV35S	Expression of active enzyme Accumulation of sesqui - terpenoid product in leaves	15, 16
2	*Nicotiana tabacum*	-	Cytochrome P450 hydroxylase gene specific to the trichome gland Involved in metabolism of exogenous and endogenous compounds	Antisense co-suppression	Transgenic plants with exudate containing high concentrations of cembratriene-ol showed greatly diminished aphid colonization responses	3, 17, 18, 19
3	*Mentha x piperita L.*	*Solanum lycopersicum*	1-deoxy-d-xylulose-5-phosphate reducto-isomerase (DXR) Catalyses the first committed step of MEP pathway	Co-suppression	Approximately 50% increase in yield	20, 21
4	*Petunia hybrid, Solanum lycopersicum, Dianthus*	*Clarkia breweri*	*S*-linalool synthase gene Catalyses conversion of geranyl diphosphate to 3S-linalool and	Constitutive over expression in flowers	Linalool and its derivatives produced by the transgenic plants act as repellents of *Myzus persicae* aphids.	22, 23, 24

	caryophyllus		diphosphate			
5	Nicotiana species	-	NpPDR1 Responsible for transportation of sclareol (antifungal compound) Strong up-regulation of *NpPDR1* in the whole leaf after infection by the bacteria *Pseudomonas syringae* pv tabaci, *Pseudomonas fluorescens*, and *Pseudomonas marginalis* pv marginalis and the fungus *Botrytis cinerea.*		Found in leaf glandular trichomes – involved in secretion of the sclareol-related diterpenes – having antimicrobial properties. No effect in roots There is volatile metabolite secretion in upper parts of petals. Involvement in induced plant defense resulting in the secretion of secondary metabolites, such as sclareol and other diterpenes, which inhibit the growth of the invading organism. Lower induction of *NpPDR1* expression seen after *Pseudomonas syringae* infection due to inhibitory effect of SA on the JA-dependent defense pathway.	25, 26, 27, 28 25, 29, 30, 31, 32 25, 33 25, 34, 35
6	*Artemisia annua*	*Artemisia annua*	Chimeric farnesyl diphosphate synthase gene	Over expression	Increase in the flux in the sesqui-terpenoid biosynthetic pathway.	3, 36
7	*Arabidopsis thaliana*	*Fragaria ananassa*	FaNES1 Responsible for production of linalool/nerolidol synthase	Plastid transformation	Produced approximately 40% - 60% higher linalool and its glycosylated and hydroxylated derivatives. Delayed growth and development *Brevicoryne brassicae* (aphid) was repelled. Diaeretiella rapae (parasitiod of aphids) preferred aphid-infested transgenic plants over aphid-infested wild-type plants for two of the accessions.	34,35

8	*Nicotiana tabacum*	*Perilla frutescens*	Perilla Limonene synthase cDNA Catalyses the stereo specific cyclization of geranyl diphosphate to form limonene	Cytosol/ plastid transformation	Limonene synthesis(monoterpene)	36
9	*Oryza sativa*	*Oryza sativa*	OsTPS3 Encodes for E-beta-Caryophyllene synthase	Over expression	Plants with increased emission rates of (E)-β-caryophyllene were more attractive to *Anagrus nilaparvatae*, an egg parasitoid of the *Nilaparvata lugens*. Attracts *Heterorhabditis megidis*, that can parasitize *Diabrotica* larvae.	37, 38
10	*Arabidopsis thaliana*	*Zea mays*	Terpene synthase gene (*TPS10*)	Over expression	Strong emission of several sesquiterpenes after herbivory by lepidopteran larvae	39
11	*Nicotiana tabacum*	*Nicotiana tabacum*	Patchoulol synthase (*PTS*) Catalyses the conversion of 2E,6E-Farnesyl diphosphate to patchoulol and diphosphate	Over expression	Synthesis of patchoulol (volatile) and 13 additional sesquiterpene products.	40
12	*Lavandula latifolia Medicus*	*Arabidopsis thaliana*	1-deoxy-d-xylulose-5-phosphate synthase (DXS) Catalyses the first committed step of MEP pathway	cDNA Transfer	Increase in yield in leaves and flowers	41
13	*Lotus japonicus* *Nicotiana tabacum*	*Phaseolus lunatus*	Terpene synthase gene – PlTPS2	Plastid transformation	Produced (E,E)-geranyl linalool and 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT). Generalist predatory mite *Neoseiulus californicus* attracted to uninfested transgenic *Lotus japonicas.* Specialist *Phytoseiulus persimilis* attracted to transgenic plants infested with spider mites.	42
14	*Arabidopsis thaliana*	*Fragaria ananassa*	CoxIV –FaNESI construct	Mitochondira/plastid transformation	Sesqiterpene precursor FPP showed increased levels in mitochondria than in plastid.	43
PHENOLICS						

15	*Lycopersicon esculentum*	*Solanum tuberosum*	Polyphenol Oxidase (PPO) Oxidises phenolics compounds and hence has anti-herbivore enzymatic activity (studied under hybrid poplar model)	cDNA transfer under constitutive action of CMV35S	Increase in oxidation of endogenous phenolic substrate which showed greater resistance against P. syringae and strong inhibition of bacterial growth.	44, 45
N-Containing Compounds						
16	*Arabidopsis thaliana*	*Sorghum bicolour*	Cyanogenic glucoside biosynthesis genes Dhurrin (cyanogenic glucoside) is synthesized from tyrosine by action of two multi-functional cytochrome P450 enzymes (CYP) and a specific UDPG-glucosyltransferase, which upon tissue damage is hydrolyzed by beta-glucosidase which releases cyanide (effective pesticide and insecticide).	Over expression of CYP79A1 (first enzyme) Over expression of both CYP genes	Synthesis of p-hydroxy benzyl glucosinolates. High levels of dhurrin (hence high levels of cyanide).	3
17	*Catharanthus roseus*	-	Tryptophan decarboxylase (TDC) and Strictosidine synthase (STR) Involved in synthesis of alkaloids	Over expression	Increased alkaloid production in case of STR but not so in TDC. Larger increase in production when fed with tryptophan and terpeniod intermediates.	46, 47, 48, 49
18	*Anisodus acutangulus* (hairy root cultures)	-	Tropinone reductase I and Hyoscyamine-6β-hydroxylase	Over expression	Enhanced production of tropane alkaloids	12

A wide variety of attempts to create transgenics with increased or new resistance against biotic stresses have been made, of which a few are explained in detail in Table 4.

CONCLUSION

Nature has gifted plants with various defense mechanisms to overcome biotic and abiotic stresses. Man has domesticated plants to serve as feed, fodder, shelter, industrial resource etc. Such plants may not exhibit the defense potential as exhibited by the wild plant varieties which grow in their natural habitat. Transgenic technology can serve as a tool in bringing in defense related genes from resistant sources and incorporating them into the cultivated plants to improve tolerance against stress.

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