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# Enhancing Production of Terpenoids in Metabolically Engineered Transgenic Spearmint *(Mentha spicata* L.) by Salt and Fungal Elicitors

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## Abstract

Forest tree species usually takes for long periods to be harvested and cultivated but spearmints are a good model system for woody plant because of reducing and shortening cultivation time. Spearmints are good model plants (*Mentha* species) for research about terpenoids production and industrial essential oil manufacture. Isopentenyl pyrophosphate isomerase (*Iso*) and limonene synthase (*Limo*) are the key enzymes of terpenoid biosynthesis pathway. Transgenic and wild spearmints (*Mentha spicata*, MS) were cultured *in vitro* and assessed for the essential oil contents. The content of essential oil of transgenic spearmint also was enhanced slightly depending on the target terpenoid genes. In an attempt to increase productivity of terpenoids further, salt and fungal elicitation strategy was adopted on transgenic *Mentha spicata*. The salt (800 mM NaCl) as abiotic and two fungi (*Botrytis cinerea* and *Glomerella cingulata*) as biotic were used for elicitors. In the absence of salt stress four terpenoids were detected from the spearmint extracts, all of them being monoterpenes. On the other hand, the transgenic (MS*Iso*) extracts contained eleven terpenoids (10 monoterpenes and 1 phenylpropene) while transgenic (MS*Limo*) extracts contained seven monoterpenes. After 3 days of fungal infection, the resistance indices further increased to 4.38, 3.89 and 2.04 for wild type, MS*Iso* and MS*Limo*, respectively. The salt and fungal elicitators proved beneficial towards modifying both the terpenoids profile and improvement in the composition of essential oil. These results have important applications for the large-scale production of essential oils and forest biotechnology with respect to spearmint.

**Key Words:** Essential oils, Elicitors, Forest biotechnology, Metabolically engineered transgenic spearmint, Terpenoids

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## Introduction

Forest tree species usually takes for long periods to be cultured but spearmints are a good model system for woody plant because of saving cultivation time. Spearmints are good model plants (*Mentha* species) for the study about terpenoids production and industrial essential oil manufacture because spearmints have the specific terpenoids biosynthesis pathway and require a short period to grow and harvest (Kang et al. 2012). Spearmint (*Mentha spicata*, MS) has been most widely cultivated in many countries of East Asia, Europe, America and Australia to meet the requirement of essential oils. Essential oil derived from *Mentha* species in general is a mixture of terpenoids in various proportions. Spearmint essential oil in particular finds application in flavoring of pharmaceuticals and oral medicinal preparations.

Spearmint is one of the main sources for aroma compounds such as; menthol, carvone, linalool, and linayl acetate used widely in food, cosmetic, flavour, and pharmaceutical industries (Singh et al. 1999). The world market for spearmint oils is approximately 1,500,000 kg/yr and increasing at approximately 5%/yr. The spearmint oil market, while smaller than peppermint oil (approx. 3,500,000 kg/yr), but is substantial and is one of the larger essential oil commodities (Bienvenu et al. 1999).

Terpenoids in plants are synthesized via the methylerythritol phosphate (MEP) pathway and non-MEP pathway, and structurally are classified as mono-, di-, and sesquiterpenoids. Like all secondary metabolites, terpenoids have also critical functions in plant defense mechanism. The instances of metabolic engineering in essential oils about terpenoids are being reported. The essential oil yield of peppermint was enhanced by the overexpression of 1-deoxy-D-xylulose 5-phosphate reductoisomerase, a MEP pathway gene (Lange et al. 2011). However, metabolic engineering of spearmint for altering terpenoid metabolites is less known.

Plant metabolism can be described as a complex network of proteins and their interacting partners. Thus, the plant metabolism is divided into primary and secondary metabolism by conventional biochemists. Generally the products of primary metabolism are considered the ones essential for plant growth and development (Bryant et al. 1991; Tegelberg and Julkunen-Tiitto 2001; Laitinen et al. 2004). On the other hand secondary metabolites are considered as the overflow-compounds (Seigler 1998). The nature of secondary compounds specific for certain plant species or tissues or they may be produced only at a certain developmental stage depending on the plant age and the prevailing environmental factors (Bryant and Julkunen-Tittto 1995; Tegelberg et al. 2001; Laitinen et al. 2002). Many secondary metabolites are also inducible as a response to wounding or pathogens (Waterman and Mole 1994; Julkunen-Tiitto et al. 1995). *B. cinerea* (Gray mold) and *G. cingulata* (Anthracnose) were used as fungal elicitors to improve secondary metabolites and fungal pathogens test for defense mechanism in plants (Jung et al. 2003; Lee et al. 2012; Choi et al. 2012).

Plant cell and tissue cultures for the production of secondary metabolites have met with limited success due to their low yields (Bryant et al. 1991). In order to improve the yield of such products, various techniques have been tried. Among the strategies employed to enhance productivity, an efficient method is the use of biotic and abiotic elicitors. The elicitation of plant cells and tissue can lead to increased yields and can shorten the fermentation time. Since the biosynthesis of secondary metabolites in plants is tightly controlled during development, the metabolites are accumulated by plants in response to stress. Thus, microbial attack is a good material to study defense response in vitro cultures. Although a number of studies have focused on elicitation to increase several metabolites, the mechanism of salt and fungal elicitors at both physical and molecular level has not yet been elucidated.

In our previously published paper, Kang et al. (2012) have introduced two genes specifying the isopentenyl pyrophosphate isomerase (*Iso*) and Limonene synthase (*Limo*) of the terpenoid biosynthesis pathway to enhance essential oil components and yields. The aims of this study were to investigate the production of terpenoids by the introduction abiotic and biotic elicitors into the environment of transgenic spearmint. Our hypothesis was that the terpenoids productivity could be enhanced by the optimal formulation of exposure time, salt concentration, and selection of fungal strains.

## Materials and Methods

### *Plant materials and construction of gene expression vectors*

Spearmint *in vitro* seedlings from sterilized seeds were prepared for general tissue culture and genetic transformation. The seeds of spearmint were provided by Agricultural farm of Gyeongsang National University. Seeds were sterilized for 15 min in 3% (v/v) NaClO and rinsed five times with sterile distilled water. The seed were placed in Petri dishes containing 20 mL of MS solid medium, supplemented with 0.1 mg/l $GA_3$ (w/v), 30% sucrose (w/v) and 0.4% gelrite (w/v). The pH of basal medium was adjusted to 5.7 before autoclaving at 121°C for 15 min. The dishes were kept in dark at 25±1°C until the seeds germinated. As well described background in previously publications (Park 2008; Kang et al. 2012), *Isoprene synthase* isolated from hybrid poplar (*Populous alba* X *P. tremula*) and *Limonene synthase* isolated from lemon (*Citrus limon*) were cloned into pBI121, which were a gift from Dr. Kim from the Gyeongsang National University. The *Isoprene synthase* and *Limonene synthase* cDNA in pBI121 were then cut with the *Xba* I and *Sal* I restriction enzymes to release the *Isoprene synthase* and *Limonene synthase* gene. Specific information about DNA manipulation, vector construction, and *Agrobacterium* transformation were carried out according to previously published methods (Mitsuhara et al. 1996; Sambrook et al. 1998; Park 2008; Kang et al. 2012).

### *Salt and fungal elicitation of spearmints*

The effect of salt stress on transgenic spearmints were determined by exposure of wild type and each transgenic spearmint (MS*Iso* and MS*Limo*) to a high salt concentration (800 mM NaCl) for 3 days. After sub-culture for 4 weeks, these were transferred to salt media. The role of terpenoids in conferring protection against pathogenic fungi was determined based on resistance of transgenics to fungal infection. *B. cinerea* (Gray mold) and *G. cingulata* (Anthracnose) were used as fungal elicitors. The Fungi were cultured in YEP liquid media under dark conditions at 24±1°C and at 120 rpm on rotary shaker for 1 week. After fungal culture, the fungal growth medium was filtered by sterilized 3M™ filter papers (3M co. Canada). The filtrate was inoculated such that it covered medium surface in culture vessels (about 4 mL) where wild type and each transgenic spearmint plant was cultured and incubated for 4 weeks. Infected plants were maintained at 25±1°C under 16 h with photoperiod for 3 days. Resistance index and elicitation were determined based on the previous study (Aoki et al. 2003; Aoki et al. 2005; Park 2008). This is a kind of morphological or observational index based on the levels of plant response after infection. The detailed resistance index was described as this (resistance index 0: Healthy shoots with all leaves green and healthy, resistance index 1: Single leaves yellowish-brown, resistance index 2: < 25% of leaves yellowish-brown, resistance index 3: 26-50% of leaves yellowish-brown, resistance index 4: 51-75% of leaves yellowish-brown, resistance index 5: 76-100% of leaves yellowish-brown).

### *Extraction and metabolite profiling of spearmint*

The terpenoids of spearmint for GC analysis were prepared adopting modified simultaneous steam distillation and extraction apparatus (SDE) method with Nickerson and Liken instrument (Nickerson and Likens 1996). Briefly, 10 g of each fresh plants and elicited plants by salt and fungi were harvested, homogenized and transferred to a steam distillation flask. Blended 25 mL ether and 25 mL n-pentane were injected into the extraction flask. Both flasks were heated on heating mantle for 1 hr. The floating organic layer of metabolites was carefully collected after enough refrigeration and using cool water. The extracts were left overnight along with sodium sulfate ($NaSO_4$) for dehydration. Finally each extract was evaporated in a rotary evaporator at 30°C under nitrogen gas ($N_2$).

Metabolite compositions of spearmint (MS*Iso* and MS*Limo*) extracts were determined through metabolite profiling with GC-MS qualitative analysis. Each plant extract was subjected to Gas Chromatograph (HP5890 SERIES II) - Mass Spectrometer (GC-MS, HP 5971 SERIES MSD) for metabolite profiling. The GC column of chromatogram was 60 mm x 0.25 mm x 0.25 μm i.d.HP-1 fused silica capillary column. The GC conditions were as follows: injector temperature, 250°C; column temperature, isothermal at 50°C for 5 min, then programmed to 240°C for 3 min and held at this temperature for 10 min; ion source temperature, 230°C. Helium was used as the carrier gas at the rate of 1 mL/min. The effluent of the GC

column was introduced directly into the source of the mass spectrometer. Mass Spectra was recorded in EI mode with 70 eV ionization energy. The sector mass analyzer was set to scan from 50 to 800 amu in 2s. The identity of terpenoids was established by comparison of the retention times obtained with authentic standards. When an authentic sample was not available, the identification was carried out by comparison of mass spectra with those available in the mass spectra library (The Wiley Registry of Mass Spectral Data, 6th ed.). The concentration of terpenoids was determined on the basis of relative area (%) of the analyzed peaks.

### *Statistical analysis*

Resistance index was determined mean with standard deviation in three replications. Differences in the means of each value were determined by one-way ANOVA followed

**Table 1.** Variation in the composition (%) of terpenoids in spearmint after salt-stress. Concentration was determined on the basis of relative percentage of peaks area on chromatography. '-' means not detected by GC/MS analysis

| Terpenoids | Components | No-Salt | | | Salt | | |
|---|---|---|---|---|---|---|---|
| | | WT | MS*Iso* | MS*Limo* | WT | MS*Iso* | MS*Limo* |
| Monoterpenes | 1,8-cineole | - | 0.25 | - | - | - | - |
| | 1-α-terpineol | - | 0.2 | - | - | - | - |
| | α-pinene | - | - | - | - | 0.02 | - |
| | α-pinene -isomer | - | - | - | - | 0.02 | - |
| | β-pinene | - | - | - | 0.02 | 0.02 | - |
| | β-pinene-isomer | - | - | - | - | 0.02 | - |
| | Borneol | - | 0.17 | - | - | - | - |
| | Camphor | - | 0.13 | - | - | - | - |
| | Carvone | - | - | - | 0.02 | 0.02 | - |
| | γ-terpinene | - | - | - | 0.02 | 0.02 | 0.02 |
| | Geraniol | - | 0.09 | - | - | - | - |
| | Isoamyl isobutyrate | | - | - | - | - | 0.02 |
| | Limonene | 0.05 | 0.1 | 0.9 | 0.02 | 0.5 | 0.59 |
| | Linalool | 0.01 | 0.07 | 0.02 | - | - | - |
| | Menthol | - | - | - | 0.02 | 0.02 | 0.02 |
| | Menthol-isomer | - | - | 0.11 | - | - | - |
| | Menthone | | - | 0.07 | 0.02 | 0.02 | - |
| | Myrcene | - | - | - | - | 0.02 | - |
| | ρ-cymene | - | - | - | 0.02 | 0.02 | 0.02 |
| | Piperitone | - | - | 0.18 | - | - | |
| Terpenoids | Components | No-Salt | | | Salt | | |
| | | WT | MS*Iso* | MS*Limo* | WT | MS*Iso* | MS*Limo* |
| Monoterpenes | Safranal | - | 0.49 | - | - | - | |
| | Terpinene | 0.04 | 0.07 | 0.05 | - | - | |
| | Terpinolene | - | - | - | - | 0.02 | |
| | Thymol | 0.07 | 0.05 | 0.66 | 0.02 | 0.02 | 0.75 |
| Sesquiterpenes | α-cedrene | - | - | - | - | - | 0.02 |
| | α-cubebene | - | - | - | - | - | 0.02 |
| | β-caryophyllene | - | - | - | - | - | 11.45 |
| | Bisabolene | - | - | - | - | - | 1.78 |
| Phenylpropene | Anethol | - | - | - | - | 0.02 | - |
| | Eugenol | - | - | - | 0.02 | 0.02 | - |
| | Methyl eugenol | - | 0.02 | - | - | - | - |

using the Statistical Analysis System software, Version 9.0 (SAS Institute, Cary, NC, USA).

## Results

### *Variation in terpenoid composition of transgenic spearmint under salt stress*

The comparative analysis of the *M. spicata* plants experiencing salt stress revealed an increase in the number of terpenoid compounds (Table 1 and Fig. 1). In the absence of salt stress four terpenoids were detected from the spearmint extracts, all of them being monoterpenes. The transgenic (MS*Iso*) extracts contained eleven terpenoids (10 monoterpenes and 1 phenylpropene) while transgenic (MS*Limo*) extracts contained seven monoterpenes. On the other hand the extracts of plants exposed to salt-stress contained nine terpenoids that could be subdivided into seven monoterpenes and a phenylpropane.

In the absence of salt stress only four terpenoids of the monoterpene type were detected from the spearmint extracts. The salt stressed transgenic (MS*Iso*) extracts contained fifteen terpenoids (13 monoterpenes and 2 phenylpropenes) while transgenic (MS*Limo*) extracts contained ten terpenoids (7 monoterpenes and 3 sesquiterpenes).

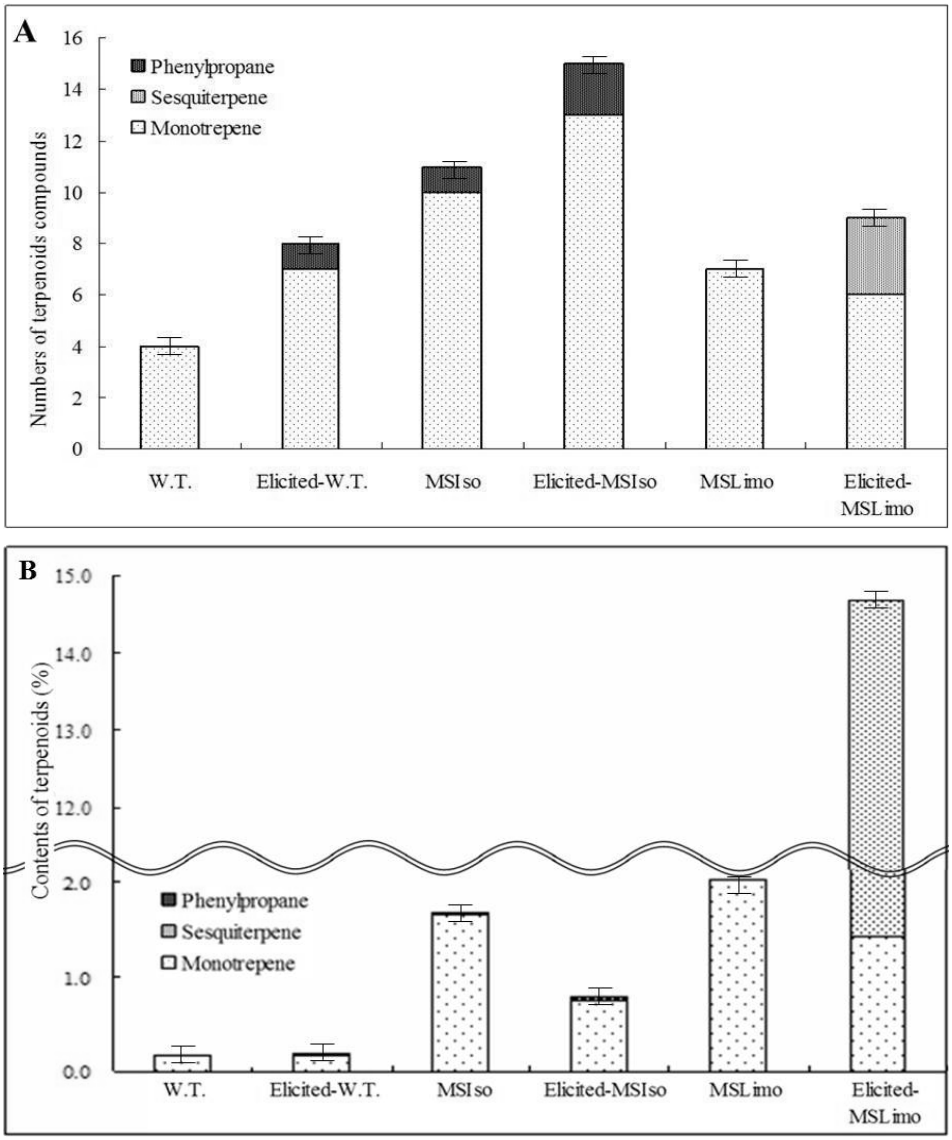


**Fig. 1.** Variations of terpenoid compositions in wild type and transgenic spearmints after salt stress as aboitic elicitation. (A) Number of terpenoid compounds composition between wild type and transgenic spearmints (B) Content of terpenoid (%) compositions between wild type and transgenic spearmints. Total content of terpenoids percentages was determined on the basis of relative percentage of peaks area on chromatography. Content of terpenoids percentage provide how much monoterpenes transfer to phenyl- or sequi- terpenes during abiotic elicitation.

### *Variations in the composition of terpenoids in transgenic spearmint after fungal elicitation*

The biological elicitation of wild and transgenic spearmint plants with filtrates of fungal cultures of *B. cinerea* and *G. cingulata* affected the metabolite composition. After 1 day of treatment with *B. cinerea* of filtrate, resistance of spearmint against *b. cinerea* are shown (Fig. 2). The resistance index of wild type was 2.66, and transgenic plants were determined to be 1.22 (MS*Iso*) and 0.34 (MS*Limo*). The wild types spearmint was damaged, but both the transgenic plants were maintained with less damage. But after 2 days following fungal treatment, the infection seemed to increase due to the fungal proliferation. After 3 days, the resistance indexes were recorded as 4.68, 4.56 and 4.34 for wild type, MS*Iso* and MS*Limo* respectively. Further it was observed that the wild type plant died, but both transgenics spearmints remained alive. Among the two MS*Limo* plants were showed higher resistance to *B. cinerea* than MS*Iso* spearmint.

Fig. 3 showed the resistance of spearmint plants to *G. cingulata* infection. After 1 day of treatment with *G. cingulata* filtrate, the damage to wild type and both transgenic plants was assessed and was shown to be similar. After 2 days of *G. cingulata* treatment, the health of spearmint plants deteriorated due to proliferation of fungus. The effects of fungal infection are expressed in terms of the resistance index. The resistance index was 2.84 for wild type and 2.72 (MS*Iso*) and 1.21 (MS*Limo*) for transgenic spearmints. After 3 days, the resistance indices further increased to 4.38, 3.89 and 2.04 for wild type, MS*Iso* and MS*Limo* respectively. The MS*Limo* plant showed highest resistance against *G. cingulata*. Furthermore, wild type spearmints were completely covered and damaged with fungal hypha of *G. cingulata* while MS*Iso* transgenic spearmints were partially damaged from fungal infection.

## Discussion

Limonene, linalool, terpinene and thynol were detected in both wild type and transgenic spearmint under control condition for all lines, but after salt treatment/elicitation only Limonene and thynol were detected among both wild type and transgenic spearmint metabolites. It seems that the production of terpenoids is obstructed by salt stress. The stress modified the production of monoterpenes like α-pinene, carvone, α-terpinene, menthol, myrcene, p-cymene, and thymol and phenylpropenes like anethol and eugenol appeared after elicitor exposure. It seems that these terpenoids are metabolites of spearmint against salt-stress, as the same were not detected under control conditions. Furthermore, MS*Iso* and MS*Limo* transgenic spearmint extracts analyzed under non-elicited condition generally produced some additional terpenoids than those presented in wild type. Although salt stressed MS*Iso* plant extracts showed similar metabolite profile, the salt stressed MS*Limo* plant extracts produced a different set of terpenoids than those presented in both transgenic lines. These results indicated that spearmint responded to salt-stress and underwent regulation of terpenoid metabolism by enhanced flux of metabolites.

α-Pinene, α-pinene-isomer, β-pinene-isomer, myrcene, terpinolene, anethol and eugenol were detected from salt stressed-MS*Iso* plant extracts, but 1, 8-cineol, 1-alpha-terpineol, borneol, camphor, safranal and methyl eugenol disappeared. It seems that these compounds are regulated by *Iso* to endure salt-stress. Thus salt-stress, influenced variations in the proportions of terpenoids compounds in MS*Iso* plant.

MS*Limo* plant when subjected to salt stress showed the occurrence of isoamyl isobutyrate, α-cedrene, α-cubebene, β-caryophyllene and bisabolene in their extracts, among these α-cubebene, β-caryophyllene and bisabolene are sesquiterpenes. However the menthol-isomer, menthone and piperitone disappeared among the metabolite profile after salt stress.

Sesquiterpenes and phenylpropenes belong to larger members in the terpenoids biosynthesis pathway. These results suggested that *Iso* and *Limo* elaborate gene products in spearmint that modify the terpenoid composition by generating high-molecular weight terpenoids under salt-stress.

The purified α- and β-pinenes are themselves flavour and fragrance ingredients (Hall and Oser 1965). However, by far the largest uses of α- and β-pinene and the two enriched fractions are starting materials in the synthesis of a wide range of other flavor and fragrance ingredients, such as linalool and geraniol, and medicinal products such as vitamins A and E.

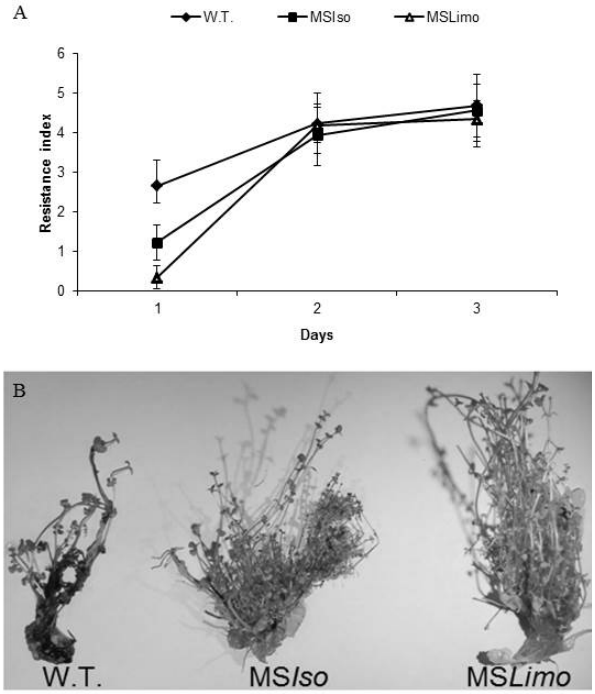


**Fig. 2.** Resistance of spearmint against *B. cinerea* infection. (A) Resistance index 0: Healthy shoots with all leaves green and healthy, resistance index 1: Single leaf yellowish-brown, resistance index 2: <25% of leaves yellowish-brown, resistance index 3: 26-50% of leaves yellowish-brown, resistance index 4: 51-75% of leaves yellowish-brown, resistance index 5: 76-100% of leaves yellowish-brown). (B) *B. cinerea* inoculated spearmint plants; wild type (W.T.), MS*Iso*, and MS*Limo* plants after 3 days.

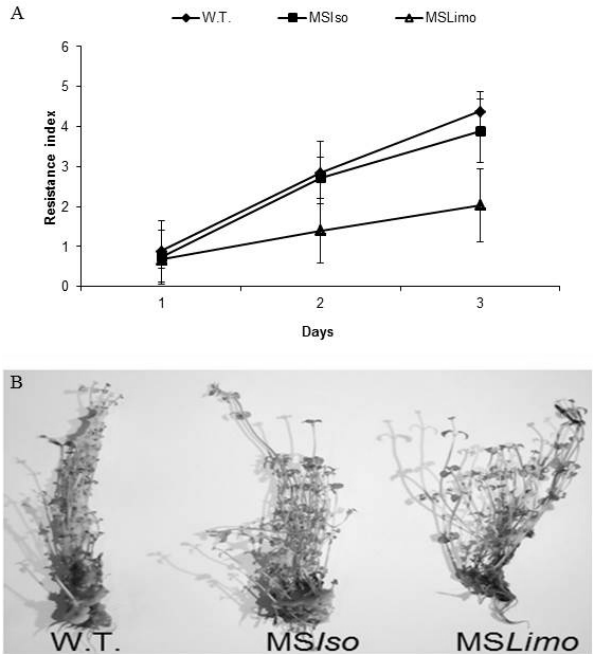


**Fig. 3.** Resistance of spearmints against *G. cingulata* infection. (A) Rresistance index (0: Healthy shoots, all leaves green and healthy; 1: Single leaf yellowish-brown; 2: <25% of leaves yellowish-brown; 3: 26-50% of leaves yellowish-brown; 4: 51-75% of leaves yellowish-brown; 5: 76-100% of leaves yellowish-brown). (B) *G. cingulata* treated wild type plant, MS*Iso* plant, MS*Limo* plants after 3 days.

Carvone ($C_{10}H_{14}O$) is the main constituent of spearmint oil, and is also a constituent of other volatile oils like those from dill and caraway seed. Carvone is also an oxidation product of *d*-limonene, the main constituent of volatile oils from some citruses. Furthermore, L-carvone has also been isolated from the volatile oil of *Chrysanthemum balsamita* and its isomer, L-carvone, from mugwort (*Artemisia vulgaris* L), both these being members of the *Compositae* family. Due to its spearmint-like odour, carvone is used as a flavouring in many products, such as candies, chewing gums, mouthwashes and toothpastes (Corazza et al. 2002). Myrcene is a naturally occurring acyclic polyunsaturated monoterpene which contains three carbon–carbon double bonds, two of them being conjugated. This monoterpene is also easily available by the industrial pyrolysis of β-pinene, one of the major constituents of pine turpentine (Erman 1985; Lawrence 1989). *p*-Cymene is also a constituent of oregano and thyme oils but is less effective against food-related pathogens (Dorman and Deans 2000; Ultee et al. 2000; Burt et al. 2005) and is thought to be a precursor to carvacrol and thymol in the plant (Ultee et al. 2002).

Terpinolene is a monoterpenic constituent of some essential oils of various fir and pine species, as well as plants such as *Manilla elemi*, *Nectranda elaiophora*, and *Dacrydium colensoi* (Burdock 1995). It displays antifungal activity against various pathogens (Himejima et al. 1992). Hence, it seemed interesting to study the detoxification of antifungal monoterpene terpinolene by plant pathogenic fungus *Botris cinerea* as an exemplary eco-chemical interaction of the aromatic plants and phytopathogenic fungi. α-Cedrene is a major component of cedar wood oil which is used widely as a perfume and as a precursor of synthetic perfumes for toiletry and cosmetic products. Abraham and coworkers reported that *Corynespora cassicola* DSM 62474 and *Rhodococcus rhodochrous* (formerly *Mycobacterium rhodochrous*) ATCC 999 converted a-cedrene to a variety of minor products, such as 3-hydroxy- and 12-hydroxy-a-cedrenes (Abraham et al. 1987) and cedrenone and 10-methoxy-a-ce-

drene, respectively (Kieslich et al. 1986). The formation of such products from a-cedrene by *Beauveria sulfurescens* ATCC 7159 was also reported (Lamare et al. 1987; Lamare and Furstoss 1990) However, the biotransformations of a-cedrene reported thus far require a long cultivation time, and the yields of products have been extremely low.

α-Cubebene is the precursor for calamenene via one of two pathways. One path requires the conversion of a-cubebene to (-)cubenol, to cadina-4,6(1) diene and then to calamenene (Menary et al. 1999). β-Caryophyllene, is a natural bicyclic sesquiterpene with a rare cyclobutane ring. β-caryophyllene is known for its anti-inflammatory and local anaesthetic activities (Tambe et al. 1996; Ghelardini et al. 2001). It is used in spice blends, citrus flavors, soaps, detergents, creams and lotions, and also in a variety of food products and beverages (Budavari 1996; Sköld et al. 2006). It has been reported as a volatile compound emitted by plants into the atmosphere in response to herbivore attack and due to change in abiotic factors (Gouinguené and Turlings 2002) and as sequestered by the Australian green tree frog, *Litoria caerulea* White from its diet (Smith et al. 2004).

Anethol is the main antimicrobial active molecule of anise oil. In general, the antimicrobial activity of anethol has been related to the ether group on its aromatic ring (Davidson and Naidu 2000). Although anethol had a high capacity to modify rumen microbial fermentation, it reduced the proportion of acetate and propionate, which are the main precursors in ruminants for fat and glucose synthesis, respectively, which suggests that anethol may not be nutritionally beneficial to dairy cattle. Eugenol is natural flavor extracts from plants. Eugenol is used as the substrate of bioconversion. Eugenol is converted under strong basic condition to isoeugenol that was subsequently transformed to vanillin by Lipoxygenase (Li et al. 2004).

In conclusion, the secondary metabolites like terpenoids increase following fungal and salt stress as biotic and abiotic elicitors. The metabolically engineered transgenic spearmint with MS*Iso* and MS*Limo* proved beneficial for general enhancements of terpenoids and their yields. The efforts undertaking the investigations on overproduction of secondary metabolites from transgenic spearmint by employing elicitation are scarce. Such an attempt offers a double advantage for enhanced production of metabolites like terpenoids. The elicitors like fungi and salt enhanced the diversity of terpenoids in MS*Iso* and MS*Limo* transgenic spearmints. Hence, the combination of genetic modification and elicitation experiments could contribute towards developing novel production technologies for enhancing the ever increasing demand for essential oils by reducing time-related processes in forest biotechnology.

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