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Induction of vanillin related compounds from nodal explants of *Vanilla planifolia* using BAP and Kinetin

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ABSTRACT

The multiple shoots of Vanilla plantifolia obtained from the nodal explant on MS medium supplemented with 6-benzylaminopurine (BAP) 2 mg l^{-1} in combination of α-Napthalene acetic acid (NAA) 1mg l^{-1} and kinetin 20 µg g^{-1} f. wt of cells were maintained by regular sub culturing every 30 days and also cultured liquid MS medium of the same combination growth regulators . Cell suspension culture of Vanilla plantifolia was used for studies on the biosynthesis of vanillic acid. Maximal induction was observed at a kinetin concentration of 20 µg g^{-1} fresh weight of cells. Cell cultures of V. plantifolia were capable of producing benzoate derivatives and enzymes involved in the synthesis appear to be present within the cells. Kinetin was used as an elicitor to induce vanillic acid formation. The induction of the few enzymes of the phenypropanoid pathway may result in the formation of vanillic acid under cell suspension cultured conditions. In vitro cultures were extracted with ethylacetate and the amount of aromatic compounds were determined in explants and compared with different developmental stages of explants were analyzed using HPLC for the presence of vanillin flavor and related compounds. This system can be used successfully to understand the biosynthesis of vanilla flavor compounds in organized shoot cultures and compared with normal plants. Explants cultured for 12 to15 weeks in MS + BAP +NAA+ Kinetin medium had attained the stage of vigorous proliferation, which were transferred for further mutiplication then plantlets were exposed to natural condition for primary hardening.

Key words: Growth regulators, HPLC, MS medium, Pharmaceutical products.

INTRODUCTION

Vanilla is an important spice crop and offers excellent scope for cultivation in the tropical high rainfall regions in southern India. The Vanilla plant is a fleshy vine which produces elongate leaves and aerial roots at the nodes. The vine or stem is green and also photosynthesizes along with the leaf. One or two aerial roots are formed at each node enabling the plant to take up water and nutrients and allowing the vine to climb on supporting trees or others structures. Healthy vines grow for many years and can reach lengths of more than 60 m. In nature, vines grow far in to the canopy of trees. Flowers are formed on short branches or racemes, with few to many flowers per cluster. Pollination requires the pollen to be placed on the sticky stigma. The fruit is a long capsule, which is known as a "bean" and when mature contains thousands of tiny black, round seeds [1].Vanilla essence, vanillin extracted from the cured beans is widely used for flavoring cakes, sweets, chocolates, ice creams, beverages in cosmetics and perfumery industries.

Vanilla is the most expensive spice material. It is also used as a fragrance ingredient. The characteristic flavor and aromatic principles are developed only after fermenting and curing of the fruit (bean) from the vanilla plant. The tropical climbing orchid is native to southeastern Mexico, and other tropical areas at the America [2]. A different species of vanilla is cultivated in Tamil and Hawaii. This vanilla characterized by its floral sweet aroma is known as Tahitian vanilla (*Vanilla tahitensis*). These beans have a peculiar sweet floral fragrance but are considered to be of poor quality for use flavorings. In the united state only the vanilla beans of *Planifolia* and *tahitensis* species are allowed in food (CFR). A sustained quantity of vanilla has been grown in Papua New Guinea (PNG) in the last five years. Vanilla beans exported from PNG have characteristics of both the *Planifolia* as well as *tahitensis* or simply a physical mixture of *Planifolia* and *tahitensis* beans [3].

Vanilla planifolia is a fleshy perennial vine. Which climbs trees or other supports by means of adventitious roots called holders [aerial roots] if not trained, it can climb 10 to 15 meters. Vanilla is exclusively propagated from cuttings. Vanilla seeds from young beans can be germinated under controlled laboratory conditions for cross breeding purpose to improve varieties. To optimize development of aroma, beans are carefully harvested, cured, and stored. During the curing process, vanillin is formed by enzymatic action on the glucosides in the beans. High quality, cured beans are at least 16 cm long, fleshy supple with slightly oily, unblemished and unscarred epidermis, whole (not split), strongly aromatic, and dark brown to almost black. Undesirable beans are hard, very soft or moldy, brown or uneven in color, weakly aromatic [8]. Vanilla cuttings with eight to ten nodes are usually used for vegetative propagation. A fertilized ovary becomes a pod which attains harvest maturity in about 8 months beans should be fully mature at the time of harvest in order to obtain quality cured beans with high vanillin content. Immature beans produce interior product, whereas over ripe beans produce split ends on the vines or during curing. The curing of green vanilla beans to obtain the well-appreciated vanilla aroma is a very laborious process.

Many fruit flavors or essential oils are synthesized by plant cell culture in an endogenous manner or are obtained from plant cells after specific enzymatic conversion during the post harvesting treatment [4]. The natural vanilla flavor is received after laborious process of cultivating vein, hand pollination, bean maturation, curing and extraction of actual flavor generating principals. Process of curing of beans using enzymes has been patented [9]. The process of curing has been employed for the first time for *in vitro* cultures of *V. planifolia*

The present study established reliable and reproducible protocol for rapid regeneration of vanilla from nodal explants using various concentrations and combinations of auxins and cytokinins. Vanilla plants are also propagated commercially via tissue culture, which has the advantage of producing material that is disease free.

MATERIALS AND METHODS

Culture maintenance

Plant material was collected from the greenhouse the SK keller & co Pvt Ltd, Green beans of *Vanilla planifolia* were purchased from Dr. K Sooryanarayana, Puttur, Karnataka. Actively growing and healthy plant shoot material of *Vanilla planifolia* were collected from the field grown plant in the morning. After removing the leaves, shoots were cut into pieces (2-3cm) each containing a single nodal region plants were thoroughly washed under running tap water for 20 minutes and with (v/v) laboline for 3 min followed by shoot segments were surface sterilized by 70% ethyl alcohol for 30 sec showed by 0.1% mercuric chloride (w/v) for 3 min and then rinsed four times with sterile distilled water. After surface sterilization explants were finely trimmed in and placed on to 30ml of basal medium consisted of MS salts and vitamins [11] supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar gel in magneta boxes. All cultures were kept at 26 ± 2^0C with 16 h photoperiod under white fluorescent light murashige and skoog's medium supplemented with different combinations of BAP (1-3 mg/ml) were used for enhancing multiple shoot induction.

Development of multiple shoots on MS medium

The multiple shoots and callus cultures obtained from the nodal explants in MS medium supplemented with BAP (2mg/l) + kinetin (20 µg/ g f.wt) were sub cultured regularly to fresh medium every 30 days. Various explants were also tried for callus initiation on the MS media containing BAP and Kinetin in different concentration and combination. These *in vitro* callus cultures were also cultured in bottle containing 5ml of liquid MS medium supplemented with BAP (2mg/l) and KN (µg/ g f.wt)on a gyratory shaker at 100 rpm. Shoots were transferred to MS basal medium for rooting.

Determination of aromatic compounds

Callus cultures, multiple shoot cultures and aerial roots of the rooted plants from solid cultures along with the cultures cultivated in liquid medium for 30 days were harvested, weighed and macerated. *In Vitro* cultures were extracted as above and amount of aromatic compounds were determined with HPLC. Spent medium was extracted with ethyl acetate, evaporated and extracts were analyzed by HPLC [6]. Quantity of vanillin and other precursors present in the cultured tissue and explants control tissues were calculated using HPLC. Vanilla flavors or essential oils are synthesized by plant cell cultures in an endogenous manner or were obtained from plant cells after specific enzymatic conversion during the post harvesting treatment. Vanillin (C_6-C_4) is biosynthesized from its phenyl propanoid precursors (C_6-C_3) and extracts of vanilla beans contain more than 250 chemicals. There are two probable pathways leading to vanillin synthesis viz, the ferulic acid and the benzoic acid pathway [5]. Ferulic acid is known as a precursor of vanillin and plant cultures are known to produce vanillin from ferulic acid. Metabolism of precursors such as ferulic acid, 4-hydroxybenzaldehyde, vanillic acid and vanillin along with bioconversion of vanillin was studied in the multiple shoot culture grown in the liquid MS medium supplemented with BAP (2mg/l) and KN (µg/ g.f.wt). All the precursors were dissolved in absolute ethanol and added to each flask at the 15th day from the subcultures at required concentration and incubated further for a period of 10 days. All the precursors were sterilized using syringe filter (Millipore) at the time of addition. Experimental culture tissues along with control tissue were harvested after 15th days and weighed (1g) subsequently crushed in blender to yield to homogenized pastes. The paste was extracted using 70% ethanol in a soxlet apparatus for 48h. For quantification of vanillin and other aromatic compounds present in the 70% ethanol extracts were analyzed by using HPLC with mobile phase 80% of 0.01 M phosphate buffer (pH 4) + 20% acetonitrile. The flow rate was adjusted to 1.0 ml/min. Amount of each component in the extract was identified by comparison of retention times with authentic sample. The standard graph was plotted of concentrations against area and the concentrations of the compounds in the test sample extracts were determined.

RESULTS

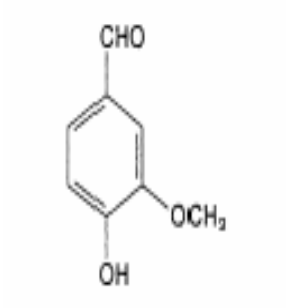
In commercial plant like *Vanilla planifolia* reliable and reproducible propagation from nodal explants is a prerequisite for genetic manipulations like transformation. The nodal explants were cultured on MS media supplemented with various concentrations of BAP 2mg l^{-1}+ KN (µg/ g.f.wt ; were sub cultured regularly to fresh medium every 30 days. The effective of nodal segments on *in vitro* micro propagation was analyzed after 30 days culture initiation. Callus induction was observed on the 12th day of incubation in all concentrations of hormones tested. The percentage of explants exhibiting callus formation was found to be 96-100 in all the combinations tested. Various explants were also tried for callus initiation on the MS media containing 2,4-D and other auxins. *In vitro* callus cultures 1g were harvested extracted using 70% ethanol (kunth and sahai 1989] in a soxlet for 48h. The amount of vanillin and other precursors present in the extracted callus tissue and control explants nodal tissues were calculated using HPLC (Table 1). This culture was established to investigate the possibility to produce vanillin in culture. However, under all the conditions tested trace extractable vanillin could be detected. The results of extraction and quantification of soluble phenolics components were summarized in table 1. Ferulic acid and 4-Hydroxy benzaldehyde were the only phenylpropanoids that could be detected in extracts of cells grown under standard conditions. Traces of 4-Hydroxy benzyl alcohol , 4-Hydroxy benzaldehyde and Vanilla glucoside could be seen transiently. However, the concentrations of these intermediate were lower indicating that they were rapidly metabolized in cultured cells. The traces of 4-Hydroxy benzoic acid , vanillic acid could be detected by HPLC after feeding cinnamic acid and ferule acid, respectively, indicates the presence of an enzymatic system capable of converting C_6-C_3 compounds into C_6-C_1 compounds within the cells. However, the concentrations of these C_6-C_1 substances are very low indicating that the fed precursors are mainly channeled in to other metabolic pathways (biosynthesis of ligneous materials). Inhibition of such competing pathways may lead to the formation of amounts of benzoic acid derivatives.

Table 1: Vanilla flavor components in the cultured tissue of *Vanilla planifolia*

Explant	4-Hydroxy benzaldehyde	4-Hydroxy benzyl alcohol	Vanilla glucoside	Ferulic acid	Vanillic acid	Vanillin
Callus culture	4.38	2.75	0.97	0.60	5.10	0.28
Regenerated shoot	1.68	3.63	1.03	0.20	4.20	0.37
Shoot in liquid medium	5.6	4.70	3.15	0.32	6.40	0.46

Note: *The valves repeated are an average of five replicates.*
All valves are expressed as µg g^{-1} f. wt .

Fig 1. Vanillin structure



Chemical Formula: $C_8H_8O_3$
Mol wt: 152.15

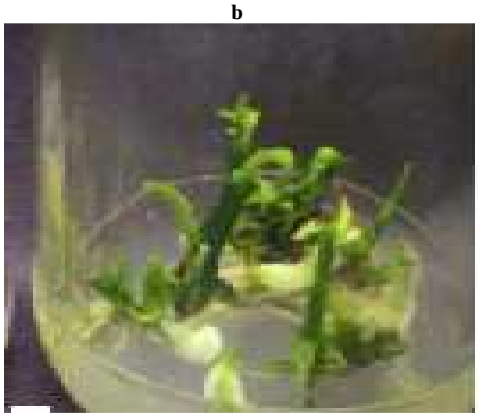
The shoot proliferation of *V. planifolia* under influence of different concentrations of BAP and Kinetin was investigated (Fig 2). Among the treatments tested, the highest number of shoots per explants (7.6 shoots) with a mean length of 4.3 cm was observed from the medium supplemented with 2.0 mg/l BAP while the PGR - free medium gave the lowest number of shoots as well as shoot length. The proliferation rate of explants cultured on the medium supplemented with Kinetin was generally lower than BAP. BAP has been considered to be one of the most effective cytokinin for the induction of shoot regeneration in plant tissue culture. Present studies showed that Kinetin was more effective than BAP in enhancing shoot multiplication on several plant species. The explants were cultured on either semi solid or liquid MS supplemented with various concentration of kinetin in combination with BAP for shoot initiation and elongation. The presence of growth hormones in both types of media has shown significantly different in the number of shoots formed per explants compared to GHR -free medium. Among the treatments tested, the liquid medium containing 2.0 mg/l BAP and Kinetin (20 µg/g.f.wt) was more effective as it induced the highest number of shoots per explants (7.6 shoots) with a mean length of 4.3 cm. High rooting response was achieved on the MS supplemented with 1.0 mg/l NAA. Multiple root development from shoot on MS containing 1.0 mg/l NAA has well established and hardened plant (Fig 2). Thirty rooted plantlets were transplanted to plastic bags containing sand and compost mixture, allowed to grow under controlled environment. The plants survival rate of 80% was recorded during hardening for one month and also it has observed an efficient *in vitro* method for rapidly inducing shoots and roots in *V. planifolia*.

In the present study metabolism of precursors such as ferule acid 4-hydroxy benzaldehyde, vanillic acid and vanillin along with bioconversion of vanillin was studied in the homogenous callus cultures grown in MS medium supplemented with BAP (2mg l^{-1}) + Kinetin (20 µg/g.f.wt) extracts of *in vitro* callus showed all the constituents such as P-hydroxy benzaldehyde, P-hydroxy benzyl alcohol, vanillic acid and vanillin in the mass of tissue of *Vanilla planifolia.*

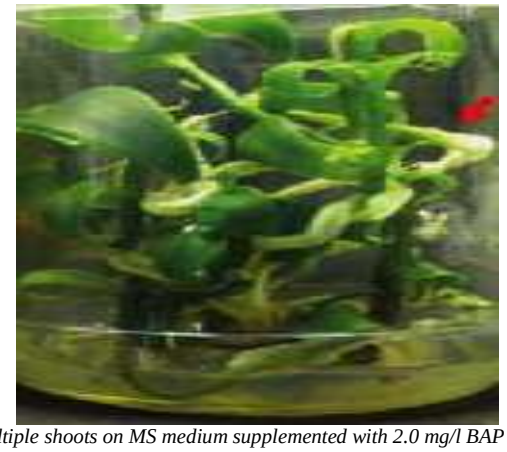
Figure 2: Regeneration of multiple shoots of *Vanillia planifolia*

a

b



c



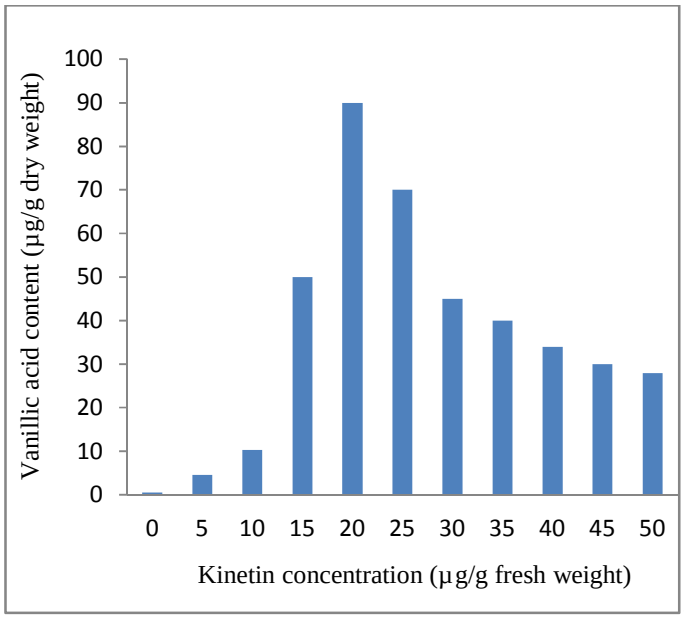
Proliferation of multiple shoots on MS medium supplemented with 2.0 mg/l BAP and Kinetin 20 µg/l
a. Initiation of shoots from nodal explants on MS medium
b. Multiple shoots development on MS medium
c. Multiple root development from shoots on MS medium

Table 2: Regeneration plantlet from in Vitro nodal explants of *V.planifolia*

S. No.	Treatment with PGR (mg/l)			Number of shoots per explant	Shoot length (cm)	Percentage of explant forming shoots
	BAP	KN	NAA			
1	1.0	0.0	1.0	2.45	2.00	40.0
2	1.0	0.5	1.0	3.02	2.30	46.0
3	1.0	1.0	1.0	3.78	2.90	49.0
4	1.0	1.5	1.0	5.25	3.82	62.0
5	1.0	2.0	1.0	5.72	4.10	66.0
6	2.0	0.0	1.0	4.90	3.49	56.0
7	2.0	0.5	1.0	8.24	6.73	90.0
8	2.0	1.0	1.0	9.47	7.80	95.0
9	2.0	1.5	1.0	7.58	7.69	88.0
10	2.0	2.0	1.0	5.01	5.32	60.0

Results represent five replicated experiments after 30days of culture.

Fig 3: Estimation of vanillic acid in cell suspension culture of *Vanilla planifolia*



Formation of vanillic acid in cell suspension culture of Vanilla planifolia as a function of Kinetin concentration. Callus cells were treated on day 10after subcultivation with various concentration of kinetin. The cells were isolated after 3days and vanillic acid was extracted and analyzed by HPLC. Time required for vanillic acid formation in culture of Vanilla planifolia has treated with kinetin. From the data, Kinetin 20 µg/g/f.wt was taken in the account of highest formation of vanillic acid.

Fig 4: Vanilla beans

Vanilla beans are long and slender with a very rich taste and smell, beans have thick oily skin containing an abundance of tiny seeds and have a strong vanilla aroma. Immature beans are uniformly dark green and tip becomes slightly yellow when the beans to ripen. To optimize development of aroma, beans are carefully harvested, cured and stored. During the curing process, vanillin is formed by enzymatic action on the glucosides in the beans. High quality cured beans are fleshy and supply with slightly oily, unblemished and unscarred epidermis strongly aromatic and dark brown to almost black in colour. The curing of green vanilla beans to obtain the well-appreciated vanilla aroma is a very laborious process. The highest grade of cured vanilla is Black vanilla and the lowest grade is Red vanilla (7). Vanillin is a natural product that can be found as a glucoside that is glucovanillin in vanilla beans at concentrations about 2%. It can be extracted with water, alcohol or other organic solvents.

Cell cultures of *Vanilla planifolia* are thus capable of producing benzoate derivatives and enzyme involved in the synthesis appear to be constitutively present within the cells. An introduction of vanillic acid formation was importance for the further evaluation of the enzymes involved in the biosynthetic pathway leading to benzoate derivatives. Growth of cells treated with kinetin ceased after addition of the regulator whereas cell treated with kinetin grew almost to the same extent as non treated cells only kinetin appeared to induces GT within the vanilla cells to great extent. A threefold induction was observed and maximum GT activity (pKat/mg protein) were

observed 24 h after addition of Kinetin. It is interesting to note that GT is only induced by Kinetin, the elicitor capable of inducing vanillic acid formation in the cells. The enzyme was induced more rapidly by Kinetin.

DISCUSSION

In the present study, the inclusion of Kinetin in the liquid MS generally showed a higher multiplication rate compared to semi solid medium (Fig 2). Kinetin has been found to be beneficial in micro propagation protocols. Several reports have also revealed the positive effects of the growth hormone supplement for the *in vitro* growth and development of different orchid species, including *Dendrobium* species, *Vanda sanderiana* and *Grandiflora* spp. (17). The effects of liquid culture in enhancing shoot proliferation are probably due to the submergence of the whole explants in the liquid medium facilitating the uptake of nutrients and growth regulators compared to semi solid media. Liquid medium based culture has reported to provide more economically beneficial method for mass propagation in a number of plant species [10]. [10] proposed that the liquid culture system could be used to save labour and expense by eliminating or reducing gelling agents in large scale *in vitro* propagation, thereby reducing the cost of micropropagated plantlets. *In vitro* derived shoots separated from multiple shoot clusters successfully developed into rooted plantlets after two weeks of culture. The results showed that the roots regeneration response in full strength MS was higher compared to MS media supplemented with NAA alone has observed to have increased in number of roots per shoot at a higher concentration (2.0 mg/l).

The effects of liquid culture in enhancing shoot proliferation are probably due to the submergence of the whole explants in the liquid medium facilitating the uptake of nutrients and growth regulators compared to semi - solid media. Liquid medium based culture has been reported to provide more economically beneficial method for mass propagation in a number of plant species (8) proposed that the liquid culture system could be used to save labour and expense by eliminating or reducing gelling agents in large scale *in vitro* propagation, thereby reducing the cost of micro propagated plantlets. *In vitro* derived shoots separated from multiple shoot clusters successfully developed into rooted plantlets after four weeks of culture.

The main component of the vanilla aroma is vanillin (4-hydroxy-3-ethoxybenzaldehyde) which then do the extraction of vanilla essence (Kroschwitz and Grant, was first isolated and identified in vanilla by Gobley et al,1858**.** In the green bean important phenolic aroma compounds are present as glucosides. The curing process is meant to release the aglycons to set free the aroma compounds. In the last century, considerable research was devoted to the vanilla curing process. Many experiments have been conducted to determine the optimal conditions to obtain a good quality of cured vanilla, the important compounds and enzymes that are involved **(15)**. Vanillin is obtained from sulfite waste liquor by further alkaline hydrolysis of lignin. The same substance can be obtained from vanilla bean extract and is the common flavoring in foods and drinks.

Vanilla flavors or essential oils are synthesized by plant cell cultures in an endogenous manner or are obtained from plant cells after specific enzymatic conversion during the harvesting. The natural Vanilla flavor is received after laborious process of cultivating vein, hand pollination bean maturation curing and extraction of actual flavor generating principles. Vanilla (C_6-C_4) and extracts of vanilla beans contain 250 or more chemicals there are two probable pathways loading to vanillin synthesis viz, the ferulic acid and the benzoic acid pathway. Ferulic acid is known as a vanillin; many fungi and plant cultures (20) are known to produce vanillin from ferulic acid. The enzyme responsible for the bioconversion process was included as 4-hydroxy cinnamoyl–coA hydratase and 4-coumarate–co A ligase were isolated and characterized from plant cell cultures (16). Vanillin and glucovanillin exhibit other biological activities such as antimutagenic agents, insect repellant and antidote for jeelyfish poison. Considering the complexity of the conventional methods of vanilla production many other alternatives suchas chemial degradation, micribial bioconversion or use of plant cell and cell and tissue are employed many of the plant cell cultures are exploited for the biotechnological production of vanillin by using immobolization, elicitation and precursor addition (18).

Pharmaceutical products

The single largest use of vanillin is as a starting material for the manufacture of an antihypertensive drug having the chemical name of Methyldopa or 3, 4-dihydroxyphenyl-2-methylalanine. L-Dopa and Trimethoprim are two other drugs that can be made from vanillin. L-Dopa is used for the treatment of Parkinson's disease. Trimethoprim is an anti-infective agent used mainly for urinary tract infections and certain venereal disease. In addition, Mabeverine that is an antispasmodic agent and Verazide, a generic anti-tubercular agent are drugs that can be made from vanillin

or its derivatives. Papeverine that is used to treat heart disease as a vasodilator is a drug that was originally made from vanillin but has since been made from veratrole and ortho-1, 2-dimethoxybenzene. Vanillin is also used as a pharmaceutical excipient (19). Vanillin is also useful as a deodorant to mask the unpleasant odor of many manufactured goods. As a masking agent for numerous types of ill-smelling mass-produced industrial products particularly those of synthetic rubber, plastics, fiberglass and inks, vanillin find extensive use In aldehylic perfumes, vanillin provides the powdery impressions given by the background smell usually up to 2% in the perfume concentrate. In cosmetics as in bath products most of the problems arising with the use of vanillin are related to the soap perfumery problems.

Food flavoring compounds

At least 30% of food-grade vanillin consumed in the world is through flavoring compounds. Flavoring compounding requires expertise to develop well-balanced and complex flavors such as fruit flavors. In the industrial production of dry cookies, cakes and pastries, the vanillin content ranges between 20 and 50 g per 100 kg of dough. Vanillin also is added during the chocolate manufacturing process in powder form in average amount of 20 g per 100 kg of the finished product. Although the vanillin concentration is a matter of taste depending on different factors in each individual case the following concentrations are generally accepted. The main application of natural vanilla is for flavoring ice creams and soft drinks. It is estimated that nearly 300 tonnes of vanilla beans is used in USA every year in the preparation of cola type drinks.

Natural vanilla is one of the most widely used and important flavoring materials worldwide. The source of vanilla is the bean or pod of the tropical Vanilla orchid that is principally *Vanilla planifolia* andrs, synthetic V. fragrance. The Aztecs of Mexico cultivated Vanilla which was then brought to Europe by the Spaniards after 1520 and is now cultivated in a number of tropical countries. The major producers are Mexico, Madagascar, Tahiti and Indonesia. Vanillin in fact occurs in trace amounts in other plants including commercial products such as tobacco. However, the pods of the Vanilla orchid remain the only commercial source of natural vanillin.

CONCLUSION

Vanilla plants are propagated via tissue culture, which has the advantage of producing material that is disease free. However, processing technology in India for vanilla is still very primitive and many farmers are satisfied with just growing and supplying green beans. Considering the fact that cost of production is low, farmers are finding vanilla beans cultivation very attractive. In future, more farmers will take up this crop and the production and export figures of vanilla will increase. Multiple shoots of *Vanilla planifolia* were induced from nodal explants under influence of different concentrations of plant growth regulators. However, propagation of in vitro cultured plantlet exhibited more amounts of vanilla flavor compounds in plantlet culture and some drawbacks such as handling of plantlet low transplant survival rate. Therefore, the present study undertaken to improve the formation of vanilla flavor contents even at its early stage of explants growth on MS medium and to evaluate the multiplication rate of *Vanilla planifolia* under the influence of plant growth regulators (Kinetin). These results provide an efficient in vitro method for rapidly inducing shoots and its aroma content. The biosynthesis of vanillic acid was induced rapidly by the kinetin treatment. It is evident that a major part of the induced vanillic acid was formed during the first day after treatment This is reflected in the enzymes for the general phenylpropanoid pathway, whereas enzymes of lignin biosynthesis(cinnomoyl CoA reductase and coniferyl alcohol dehydrogenase) were induced and enzyme potentially involved in benzoate biosynthesis (O-methyl transferase and GT) had induced by kinetin only. The induction of vanillic acid formation by kinetin appears to be linked to the induction of a benzoate biosynthesis enzyme may be considered to be a key step of phenylpropanoid metabolism in cells of *Vanilla planifolia* .This assumption was supported by previous findings that isoferulic acid when fed is converted by the culture to vanillic acid. The results show the ability of multiple shoot cultures to take up externally added ferule acid and vanillin. Curing of explants and cultured tissues may be optimum for some enzymatic conversions that resulted in the changed pattern of vanilla flavour components. The system can be used successfully to understand the biosynthesis of vanilla flavour compounds in organized shoot cultures and compared with normal plants.

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